STUDIES ON RENAL FUNCTION IN THE COW WITH PARTICULAR REFERENCE TO BICARBONATE EXCRETION

Thesis Submitted for the Degree of

Doctor of Philosophy

of the University of Glasgow

by

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May, 1964.

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Studies on Renal Function in the Cow

With Particular Reference to Bicarbonate Excretion

The absence of any quantitative studies to support the generally held belief in the causal relationship between the "alkaline ash" diet of the cow, and the excretion of an alkaline urine prompted a study of the excretion of bicarbonate by the dairy cow.

Initially techniques were developed and tested for accurate measurement of urine flow and glomerular filtration rate.

The factors affecting the collection and storage of bovine urine samples for the estimation of bicarbonate concentration and pH were examined. The changes in bicarbonate concentration and pH occurring on an exposure of samples to air were recorded and a method of anaerobic collection described.

The diuretic response of cows to a painful stimulus was recorded. The effect on urinary bicarbonate concentration and excretion rate was examined, and these results compared with the renal responses of other species to painful stimuli.

The use of local and regional anaesthesia during urine collection was investigated, and cystometric measurements of bladder pressure were made. It was shown that pressure in the bovine bladder was usually sub-atmospheric (-5 to -10 cm H_2O).

The/

The "first appearance time" in the bladder of a solution of phenol red injected into the jugular vein was found to be 162 secs.

The technique of continuous collection of urine from the cow was described and its accuracy assessed.

A single injection method for measurement of glomerular filtration rate (inulin clearance) was found to be unreliable, and a constant infusion technique was developed. The mean glomerular filtration rate of 103 determinations on 7 cows was 1,100 ± 236 ml/min/500 Kg.

A survey of urinary bicarbonate concentrations and pH values was carried out on a herd of Ayrshire dairy cows. The mean bicarbonate concentration was 131.0 ± 56.3 mM/l and the mean pH was 7.92 ± 0.21. There was no significant difference between the values obtained when at grass, or indoors, nor was there a significant difference between groups of lactating and non-lactating, and pregnant and non-pregnant animals. The mean calculated pCO_2 of all samples was 64 ± 22 mm Hg. The relationships between pH, pCO_2, and bicarbonate concentration were the same as ~~that~~ Those observed in dogs subjected to experimental sodium bicarbonate infusion.

Arterial blood was sampled anaerobically during urine collection in 60 experiments on 12 cows. The mean total CO_2 concentration in the plasma was 29.1 ± 2.2 mM/l. (Range 26.2 - 33.0) and the mean excretion rate of total CO_2 was 1.38 ± 0.73 mM/min (Range 0.05 - 4.28). There was no over-all relationship between plasma total

CO_2/

CO_2 concentration and the urinary excretion of total CO_2.

Bicarbonate clearance measurements were carried out during inulin infusion in 20 experiments on three cows. The mean rate of bicarbonate reabsorption in each cow was (2.90 ± 0.19, 2.67 ± 0.15, and 2.39 ± 0.12) mM/100 ml glomerular filtrate, while the corresponding arterial plasma bicarbonate concentrations were (29.4 ± 2.1, 27.2 ± 1.1 and 25.0 ± 1.8) mM/l. There was a significant proportional relationship between the plasma concentration and reabsorption rate. The mean arterial pCO_2 was 44 (Range 43 - 45) mm Hg in the three cows. Bicarbonate excretion rate was (0.039 ± 0.029, 0.113 ± 0.049 and 0.110 ± 0.067) mM/100 ml. glomerular filtrate, and was not related to plasma bicarbonate concentration. There was, therefore, a bicarbonate 'leak' into the urine at all plasma concentrations between 21.9 and 32.8 mM/l.

The excretion of sodium and bicarbonate was enhanced by a carbonic anhydrase inhibitor, acetazolamide. A less marked kaliuresis occurred in the cow than has been observed in man and the dog. A fall in glomerular filtration rate occurred after administration of acetazolamide. Hydrochlorothiazide caused a natriuresis and chloruresis but did not show a carbonic anhydrase inhibiting effect at the doseage used. Both drugs caused an increase in the rate of urine flow.

The/

The following conclusions were drawn from the results of this study.

As the mean bicarbonate concentration of arterial plasma in the cow (27.5 mM/l) is close to the upper limit of the normal range in man (26 - 28) mM/l. the invariable presence of bicarbonate in bovine urine is partly related to a high plasma concentration. A marked bicarbonate 'leak' occurs into bovine urine, however, at all plasma levels between 22 and 33 mM/l, while in man bicarbonate is virtually absent from the urine at plasma levels below 25 mM/l.

Of the factors known to alter the inter-relationship between plasma bicarbonate concentration, and renal reabsorption, and excretion, it appears that the most likely explanation for the constant presence of bicarbonate in bovine urine is the high rate of potassium intake, and excretion.

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MAY, 1964

P R E F A C E

Chez les herbivores et chez les carnivores l'urine présente des différences sensibles; mais ces différences tiennent non à l'espèce des animaux mais à la nature de leur alimentation.

Claude Bernard (1859)

Since Bernards observations, renal responses to variations in diet or to other factors tending to alter the constancy of the 'milieu intérieur' have been the subject of extensive study. Most of the work has been carried out on dogs in which the responses to dietary and other variations are usually similar to those of man. Studies on renal function in herbivores are comparatively scarce, partly because of the dissimilarity of their homeostatic requirements to that of man, but also due to the incompatibility of large domestic herbivores with physiology laboratories, and to the apparent absence of any economically important disease problems directly associated with renal dysfunction.

The well-tried techniques of renal investigation in man and the dog have infrequently been modified and applied to herbivores, and, in contrast to the

detailed studies in man and the dog, there are few records of the use of these techniques in the investigation of metabolic diseases of extra-renal etiology in cattle. It is nearly a century since Walter (1877) showed that rabbits succumbed to relatively smaller doses of acid than did dogs, but subsequent verification, or refutation, of this significant report has not been reported, nor has its pertinence to metabolic disease in herbivores received attention.

Examination of the literature pertaining to renal regulation of acid-base balance in man and the dog showed that one of the most fruitful studies was that of bicarbonate excretion. Bicarbonate is the principal buffer-base in mammalian extra-cellular fluid, and the renal regulation of its plasma concentration has been shown - in a series of elegant experiments by R.F. Pitts - to be a fundamental factor in the maintenance of homeostasis. This aspect of renal function in the cow had not been investigated before, and was therefore selected as the subject for study in the present work.

The writer is aware that the ensuing study is only the first step in investigation of the largely unexplored field of renal responses to disturbances of

electrolyte balance in herbivores. The results have posed many more questions than they have answered, and there is every reason to continue and expand fundamental and applied studies on renal function in cattle.

A C K N O W L E D G E M E N T S

It is a pleasure to express my thanks to those who helped and influenced me during these studies.

I am grateful to Professor W.L. Weipers for the opportunity to carry out this research, and for his interest and support throughout its course.

My thanks are also due to Dr. J.W. Black for his enthusiasm and encouragement when they were most needed, and to Professor W. Mulligan and Dr. A.F. Munro for their advice in the preparation of the manuscript.

I am also indebted to Dr. T.A. Douglas for his constructive interest and for carrying out plasma protein and chloride estimations, and to Mr. A. Finnie for his impeccable photography.

My deep gratitude is due to my friend Mr. Eric Pickering with whom it was a pleasure to be associated during much of this work. The work described in Part I, Section 4, and in Part II, Sections 3 and 4 was joint in so far as both the author and Mr. Pickering carried out joint and simultaneous experiments on a single animal.

Finally, I wish to express my thanks to the Animal

Health Trust for their award of a Research Scholarship for the first two years of my studies, and to the Agricultural Research Council under whose financial support a large part of this work was accomplished.

C O N T E N T S

PART II

A STUDY OF THE RENAL EXCRETION OF BICARBONATE IN COWS

G E N E R A L I N T R O D U C T I O N

The foundations of current concepts of renal physiology on which the present study is based were laid between 1920 and 1940. Development of two techniques - sampling of glomerular and tubular fluid by micro-puncture, and its micro analysis, and the use of inulin clearance as a measure of glomerular filtration rate - substituted measurable and interpretable results for what had previously been surmise and hypothesis.

The stimulus for quantitative rather than qualitative studies on renal function was, however, largely attributable to Cushny's (1917) first monograph on the secretion of urine. At this time, physiologists were divided in their theories on urine production, and both camps based their concepts of function on anatomical rather than functional studies. Bowman (1842) and Heidenhain (1874) and their adherents believed that the formation of urine resulted from the 'vital activity' of the glomerulus and tubules, that 'the glomerular capsule secretes water, and those salts which accompany water everywhere in the organism, such as sodium chloride.' The epithelium of the renal

tubules was credited with the addition of other substances such as urea by further secretion into the tubular fluid. As Cushny (1926) pointed out, this hypothesis was mainly remarkable for its defensive strength in that every possible experimental finding could be attributed to some special activity of unspecified cells. It offered, however, no point from which advance could be made, and its advocates contented themselves with demonstrating the shortcomings and failures of other views of renal activity without attempting to define their own position more clearly.

Shortly after the Bowman theory was published, Ludwig (1844) put forward his concept of urine formation. This was based on the belief that the capsule of the glomerulus acted as a simple filter which permitted the passage of all plasma constituents except protein. This fluid was then modified in its passage through the tubules by the back-diffusion of water and other constituents by a simple physical process.

Cushny sifted through some 6000 pages of published matter related to renal function in the course of preparing his first monograph on renal function. By extracting only the ascertained facts

from the lengthy and involved discussions, he compounded a hypothesis which he called 'The Modern Theory.' This differed in some respects from both the Bowman - Heidenhain, and the Ludwig theories, but utilised the experimental findings of both to establish a more credible concept. Cushny was an opponent of the old vitalistic theories of renal function for which there was then still some support, and in presenting his monograph said that 'If it serves as an advanced post from which others may issue against the remaining ramparts of vitalism, its purpose will be attained.' The 'Modern Theory' proposed that the secretion of urine was accomplished by two distinct processes differing from each other in site and nature. The first of these upheld the view of Ludwig that filtration took place at the glomerulus, and was purely a physical process. The second process was the reabsorbtion of filtrate from the tubules and was the result of active reabsorption by the epithelial cells. The fluid thus returned to the blood was a 'perfected Lockes fluid which returned useful constituents to the blood at optimal concentrations, and allowed the rest to escape in the urine.'

Cushny's death in 1926 before the publication of the second edition of his monograph denied him the

knowledge of the advances made in this field in the following ten years. Shortly before his death he had met A.N. Richards who in 1924 began his series of studies of the tubular fluid of the frogs kidney by a micro-puncture technique. By use of this technique, and the subsequent development of the appropriate micro-analytical methods, Richards and his collaborators were able to collect and analyse capsular and tubular fluid in various parts of individual nephrons. This work confirmed much of Cushny's 'Modern Theory', demonstrating that glomerular fluid was essentially protein-free, but contained glucose, despite the fact that bladder urine was glucose-free. (Wearn & Richards, 1924).

About this time Marshall (Marshall & Vickers, 1923) published a paper on the secretion of phenolsulphonphthalein by the kidney which purported to prove the secretory ability of the convoluted tubules. Though this work was not accepted entirely by many workers, Marshall's (1931) subsequent experiments with the same substance provided convincing evidence of tubular secretion.

Further progress in the field of quantitative studies of rates of filtration, reabsorption and secretion of various substances was hindered by the

absence of a substance to act as a standard of reference, to be completely filterable by the glomerulus, and neither reabsorbed nor secreted by the tubules. The events leading up to the discovery and use of inulin to meet this need were described by Homer Smith (1949) in his Porter Lectures, and the criteria upon which inulin clearance has been judged as a measure of glomerular filtration rate have been defined by the same author (1951). Once methods of measuring glomerular filtration rate by inulin clearance had been established, quantitative studies of tubular reabsorption and excretion were at last possible, and the use of the concept of renal clearance of inulin is still an essential tool of the renal physiologist. The subsequent discovery that the clearance rate of diodrast and para-amino-hippurate was equivalent to the rate of renal plasma flow put ancillary tools at the physiologists' disposal, and in recent years, the development of stop-flow techniques, and more sophisticated surgical, micro-puncture and micro-analytical procedures have further increased the methods available for renal function studies.

A more detailed revue of the various methods of measuring glomerular filtration rate by inulin clearance in different species is contained in Part I,

sections 3 and 4 of this thesis.

The understanding of almost all aspects of renal function have benefited by the application of clearance techniques, and a much clearer concept of fluid and electrolyte balance has emerged. In particular, the studies of R.F. Pitts on the role of the kidney in the maintenance of homeostasis have advanced understanding of acid-base balance. For the last twenty years the steady output of renal research from Pitts' laboratory has been based on the concept of renal clearance and the utilisation of inulin as a measure of glomerular filtration rate. His findings and interpretations on renal bicarbonate excretion in the dog (Pitts & Lotspeich, 1946) and in man (Pitts, Ayer & Schiess, 1949) have remained unchallenged despite the attention of many subsequent workers to this aspect of renal function. This present study owes more to Pitts in its experimental approach and appreciation of renal acid-base regulation than to any other single worker.

It has long been known that the participation of the herbivore's kidney in maintaining water and electrolyte equilibrium is rather different from that of the carnivore or omnivore. Bernard (1859) observed that the reaction of herbivores' and carnivores' urine was alkaline and acid respectively,

and that this difference was related to the dietary habits of the individual species rather than to any other species characteristic. By reversing the normal dietary regimes of rabbits and dogs, he also reversed the reaction of their urine. It is debatable whether his rabbits faced with a diet of boiled beef '..... which they eat very nicely when they are given nothing else'. (Bernard, 1878), might not have exhibited a starvation ketosis rather than the excretion of a true carnivore-type urine. Bernard's earlier observations (1859) however suggest that he was aware of the effect of starvation on urine reaction. He also found that the reaction of urine did not depend solely on whether the food was of vegetable or animal origin, but that herbivores fed on a vegetable diet of high protein content (oats) excreted an acid urine. This relationship between the chemical constituents of the diet and the reaction of the urine was clearly in accord with his vision of a constant internal environment in all mammals, and still remains as the foundation stone of current views on fluid and electrolyte balance. MacCallum (1926) extended Bernard's concept into the realms of palaeochemistry, and related the present internal environment of the mammalia to that of a common protovertebrate ancestor of the Palaeozoic seas

some 500 million years ago. To preserve their existence, and maintain their independence from the vagaries of their external surroundings, succeeding generations developed an impermeable integument, thus enclosing and perpetuating fluids approximating more closely to the ancient seas than to those of the present day. The attainment of independence created internal problems, and the evolution of the kidneys as excretory and regulatory organs was essential to the solution of one problem - the maintenance of a constant internal environment in the face of variations of diet and metabolic requirements. With the further evolution of species, there occurred some adaptation of internal organs in response to the animals dietary habits, among these the development of the visceral organs of the ruminant in response to its bulky fibrous diet. Functional adaptations of the kidney to environment have been shown by the Schmidt - Nielsens and their co-workers (1948 a, b; 1957; 1958 a, b; 1961) in a number of mammals. Their findings suggested the possibility of some difference between the renal function of the herbivore and that of the carnivore.

The general acceptance of the alkalinity of herbivore urine, despite the apparent similarity of

plasma electrolyte concentration seemed, therefore, to pose the problem of whether in fact this was a result of a functional adaptation of the kidney of the cow, or whether it was a regulatory response to the requirements of electrolyte balance which was explicable in terms of existing knowledge of renal function in other mammals. The urinary pH values of cattle quoted in standard works of reference are greater than 7.0, (Dukes, 1955; Sippel, 1963) and most workers have found values between 7.0 and 8.4 (Ashworth & Brody, 1933; Galloway, 1936; Szolnoki, 1941; Poulsen, 1957; Barrada, 1957). The explanation of this alkalinity is generally attributed to the requirement of the cow to excrete the excess of inorganic cations - primarily potassium - in the diet. The organic anions accompanying the abundant potassium in vegetation and vegetables are metabolised, yielding bicarbonate to accompany the excess potassium in the urine (Dukes, 1955). Thus, the ingestion and subsequent metabolism of a vegetable diet has the same effect on the reaction of the urine as the ingestion of bicarbonate.

Despite this generally believed, and often reiterated explanation of the alkalinity of herbivores urine, there is little evidence, other than that from experiments on dogs and man to support this hypothesis.

There are comparatively few renal clearance studies in cows, and none of these have been concerned with the renal control of bicarbonate excretion.

That the acidity or alkalinity of the urine is not simply a result of ingestion of a meat or a vegetable diet was shown by Hunt (1956). In experiments with human subjects he refuted the prevalent impression that people living on vegetable diets, (oranges, potatoes, beans, raisins, apples, bananas, beets etc.) have an alkaline urine in contrast to those living on a mixed diet. He cited Bodansky (1938) who described vegetables as base-forming, this being dependent on the alkalinity of the ash of the diet which can be calculated as described by McCance and Widdowson (1942). Hunt's experiments showed that persons living on a diet with an alkaline ash rarely excrete an alkaline urine. As Hunt did not use anaerobic precautions in the collection of his urine samples, the true values were probably even more acid than recorded. Although these experiments on vegetarian human subjects permit no conclusions about the ruminant herbivore, they do give grounds for some scepticism of the generally held concepts of the alkalinity of herbivore urine.

Examination of the original article (Ashworth & Brody, 1933) from which pH values in bovine urine were

quoted (Dukes, 1955) showed that no anaerobic precautions were taken in the collection of urine samples. With the exception of Szolnoki (1941) the values recorded by other authors (v.s.) were also obtained from urine which had been exposed to the atmosphere, often for several hours. Szolnoki, whose work has not been translated from the original Hungarian, collected samples with and without anaerobic precautions and found that those exposed to air had a higher value than those collected under oil. As it has been known for some time (Marshall, 1922) that exposure of urine to air could cause marked variations in pH due to CO_2 loss, it seemed likely that pH values usually quoted for bovine urine were higher than the true values.

As experiments in the dog (Pitts & Lotspiech, 1946) and man (Pitts et al. 1949) had shown that bicarbonate was always present in alkaline urine and was indeed directly related to urinary pH, it was not unreasonable to expect that bovine urine would contain a considerable concentration of bicarbonate. There were, however, remarkably few reports of urinary bicarbonate measurements in cattle. Two articles (Brouwer, 1935; Dale, Goberdahn & Brody, 1954) contained records of urinary bicarbonate values, but

these measurements were made under abnormal dietary conditions, and collected without anaerobic precautions. No report of normal values was found. Though several authors have investigated the urinary excretion of electrolytes in normal and abnormal cattle (Sellers & Roepke, 1951 a, b, c; Knudsen, 1960; Vogel, 1962), none of these included bicarbonate values in their reports.

The exhaustive studies of Pitts on bicarbonate excretion have been collated in his recent book on renal physiology (Pitts, 1963) from which the following information was abstracted. If, in man, the plasma bicarbonate concentration is lowered by ingestion of ammonium chloride, all the bicarbonate filtered through the glomeruli is reabsorbed, and none is excreted until the plasma level attains a value of 26 - 28 mM/l, the so-called 'bicarbonate renal threshhold'. As the plasma concentration increases above 28 mM/l, a limited amount of bicarbonate, equal to 2.8 mM/100ml or 28 mM/l of glomerular filtrate is reabsorbed. Reabsorption in the dog is essentially similar except that the threshhold is slightly lower, 24 - 26 mM/l, and the transport rate slightly less, 2.6 mM/100ml, or 26 mM/l of glomerular filtrate. Under normal conditions, the plasma concentration in

man is poised at a value slightly below the renal threshhold and thus there is normally little bicarbonate in the urine. If, however, the plasma concentration of bicarbonate were to exceed the threshhold owing to the ingestion of bicarbonate or the metabolism of the salts of organic acids, the continued reabsorption of bicarbonate at a rate of 26 - 28 mM/l of filtrate, and the excretion of the excess would gradually lower the plasma and interstitial fluid concentration to the normal range. Excretion would then cease. If there was always an excess of inorganic cations in the diet, as there is in herbivorous animals, the processes of reabsorption and excretion alone would serve to stabilise the concentration in body fluids within the usual limits of normal.

It is essential in establishing the relationship between bicarbonate excretion and its plasma concentration that the plasma samples be from arterial blood. In studies of the renal clearance of other electrolytes, venous blood concentrations are almost identical to those in the arterial blood and venous concentrations may be used for clearance calculations. There is however, an arterio-venous difference in bicarbonate concentration of about 1.6 mM/l (Dittmer

& Grebe, 1958) and consequently venous samples are not representative of the plasma filtered at the glomeruli. The only figures available for total CO_2 concentrations in bovine arterial plasma are those of Fisher (1959). He found that in normal cattle the bicarbonate concentration of arterial plasma was the same as that of man. Thus, existing information suggested that the 'bicarbonate renal threshhold' in the cow must be lower than that in man.

There are at least four factors which may influence the threshhold of bicarbonate excretion. These are (1) Changes in the pCO_2 of arterial blood (Schwartz, Falbriard & Lemieux, 1959) (2) Variations in the plasma level of chloride (Pitts & Lotspeich, 1946) (3) Variations in the body store of potassium (Fuller, MacLeod & Pitts, 1955) (4) Variations in the secretion of the adrenal cortical hormones, (Giebisch, MacLeod & Pitts, 1955). Thus the threshhold for excretion is not constant. It varies between man and the dog, and it may be varied by specific requirements for homeostasis.

The present study has been concerned with the development and assessment of methods of measuring glomerular filtration rate in the cow, and the use of the techniques developed for examining some aspects of bicarbonate filtration, excretion and reabsorption by

the bovine kidney. The work is described in two parts, each of which is subdivided into sections. In Part 1, each section incorporates an introduction, description of methods, results, discussion and summary. There is short general description of the management of cattle as subjects for physiological research, and the overall findings are incorporated in the general discussion, summary and conclusions.

MANAGEMENT OF EXPERIMENTAL ANIMALS

With the exception of a survey of urinary pH and bicarbonate values in a dairy herd, experiments were carried out on small numbers of animals, the findings being compiled from many experiments on a few individuals. Though such a policy has obvious disadvantages in analysis of results, the author believes that it was justified in the present work, and is indicated in similar studies. Both Knudsen (1960) and the author (1961) have emphasised the effect of stress on cattle undergoing renal clearance experiments. Major variations in renal function may occur in the cow as a result of painful or disturbing influences. More reliable results may, therefore, be obtained from a few tranquil animals accustomed and trained to the experimental procedure than from large numbers of animals without such previous experience.

In all the experiments to be described, the animals were handled quietly at all times, and pain or discomfort was minimised wherever possible by the use of local or regional anaesthesia. When a newly purchased animal appeared fractious during experimen-

experimentation, it was sold, and replaced by another animal of more equable disposition. Only dehorned cattle were purchased to minimise the risk of damage to equipment and personnel. The early practise of dehorning experimental animals in the hospital was abandoned because of the long delay necessary between dehorning and experimentation. Recently dehorned cattle tended to be hypersensitive to manipulation of the head and neck.

Animals were brought from the field or byre on the morning of the experiment and placed in the stocks (Fig. 16). Catheterisation of the jugular veins was carried out under local anaesthesia with the animal firmly restrained by the nose. Thereafter restraint was reduced to the minimum, the head being loosely held by a halter, and wide stocks preventing excessive movement. Experiments lasted for 4 to 6 hours, and water, but not food was available during this period. Hydration, as commonly practised in renal investigation in man and the dog was not carried out in these experiments, but all animals had access to unlimited water before the experiments. Unless otherwise specified, cows were always clinically healthy, non-pregnant, non-lactating and the diet was grass or hay and concentrates.

As a result of the precautions taken to minimise pain and discomfort, it was gratifying to note that animals under experimentation frequently ruminated, and rarely manifested signs of agitation.

Detailed descriptions of experimental methods and procedures are described in their appropriate sections.

PART I

A STUDY OF THE TECHNIQUES OF RENAL FUNCTION MEASUREMENT IN COWS

Section I

Factors affecting the collection and storage of bovine urine samples for CO_2 and pH analysis

Factors affecting the collection and storage of bovine urine samples for CO_2 and pH analysis

In order to obtain reliable data on bicarbonate concentration and pH of bovine urine samples, the precautions necessary to prevent errors arising from sampling and storage technique were examined. Human and canine urine is known to lose CO_2 on exposure to air, thus altering pH and total CO_2 values (Marshall, 1922; Gamble, 1922). Where measurements of these constituents in man and the dog are carried out, therefore, suitable precautions to minimise CO_2 loss are taken - urine usually being collected under mineral oil (Pitts & Lotspeich, 1947). Despite these observations in man and dog, most of the published data on pH values for bovine urine has been obtained from samples collected without such precautions. (Ashworth & Brody, 1933; Galloway, 1936; Barrada, 1957; Poulsen, 1957).

Szolnoki, (1941) noted that the mean pH of bovine urine samples was markedly higher when collected without anaerobic precautions, than when collected by

catheter, but his work has not been translated from the original Hungarian.

The bicarbonate-carbonic acid buffer system is particularly influential in alkaline urine (Gamble, 1922). Its relationship to pH is expressed by the Henderson Hasselbalch equation:-

$$pH = pK' + \log \frac{[HCO_3]}{[CO_2]}$$

$$pH = -\log_{10} H^+$$

pK' = $-\log_{10}$ times a composite constant including the first dissociation constant of H_2CO_3 and the activity coefficient of $NaHCO_3$.

Despite the fact that less than 1% of dissolved CO_2 is as carbonic acid (H_2CO_3), the whole of the CO_2 in solution is often referred to as H_2CO_3, by convention. Thus, reference to a carbonic acid concentration of 1.4 mm/l in blood or urine means, in fact, a dissolved CO_2 concentration of 1.4 mm/l, of which a tiny fraction is as H_2CO_3. (Robinson, 1961).

CO_2 and water are in equilibrium within H_2CO_3.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

and thus, loss of CO_2 from any solution containing dissolved CO_2 lowers the H_2CO_3 concentration and elevates pH.

When a gas dissolves in a liquid, the

concentration of the gas in the liquid is directly proportional to the partial pressure of the gas, thus,

$$(\text{Dissolved } CO_2) = \alpha\ pCO_2$$

where α = a proportionality constant.

If biological fluids of high pCO_2 are exposed to air (pCO_2 = 0.2 mm Hg), then the pCO_2 of the fluid will equilibrate with that of the air, and the resulting CO_2 loss will lower the concentration of dissolved CO_2 and hence elevate the pH. In biological solutions like blood and urine, pH changes are modified by the presence of other buffers such as protein and phosphate.

Marshall (1922) found that the pH change of human urine in exposure to air was marked in dilute urines where the buffer capacity was inefficient, and that there was a greater loss of CO_2 from alkaline, than from acid urines. At this time both Marshall and Gamble (1922) believed that the concentration of dissolved CO_2 in urine was similar to that of blood, whereas it has since been shown that the dissolved CO_2 in urine of alkaline pH may be higher than that of blood (Pitts & Lotspeich, 1946). This later finding makes it easier to understand why there is a rapid loss of CO_2 from alkaline urine.

In the present investigation an attempt was made to answer the following questions:

(1) Is the urine of the cow susceptible to similar changes on exposure to air as human urine?

(2) What are the optimum conditions for the collection, transport, and preservation of bovine urine for total CO_2 and pH measurement?

METHODS

Samples were collected from normal cows either in the Field Station or in the Veterinary Hospital. Restraint was exerted by one man at the animal's head and/or one man holding the tail erect. A sterile Nielsen Catheter with a 30 in. extension of polythene tubing was used for urine collection. This extension tubing was clamped off during catheterisation to avoid introduction of air into the bladder.

The perineal area was thoroughly cleaned and swabbed with antiseptic before catheterisation, and the catheter, vulva and operator's hands lubricated with water soluble lubricating jelly*. The external urethral orifice was located with the second finger of the left hand, and, with the finger in the orifice and the hand supinated, the catheter was slipped along the palmar surface of the hand and second finger through the urethra and into the bladder. An empty 20 ml. syringe was then applied to the free end of the tubing

* 'KY', Johnson & Johnson.

and, having removed the clamp, siphonage was induced by withdrawing urine from the bladder into the tubing with the syringe. During collection, the free end of the tubing was kept at least 2 feet below the vulva to maintain the head of siphonage. Once the urine was flowing freely, two 50 ml. centrifuge tubes were filled. In one, containing 5 ml. of liquid paraffin, the tip of the collection tubing was introduced below the level of oil and the tube filled to the brim. The tube was then sealed with a rubber bung, thus displacing most of the paraffin and all the air. The other tube was empty, and the urine was allowed to run freely into it until almost full. This tube was stoppered during transport but unstoppered while on the bench. Both groups of tubes were transported in Thermos flasks containing ice (Peters & Van Slyke 1932). Total CO_2 estimations were carried out one to four hours after collection. The pH was measured within an hour of sampling, and again four or five hours later. In an extension of this experiment, a few of the tubes were stoppered and allowed to stand at room temperature for about fifteen hours to assess the change in total CO_2 concentration to be expected over this period of time. As urine samples were occasionally cloudy, a further experiment to show the effect of centifugation on total CO_2

concentration was carried out. Each anaerobically collected urine sample was divided into two by transferring half the sample from one tube to another without exposure to air, and stoppering. One tube of each pair was then centrifuged for 30 minutes at 3,000 r.p.m., and the total CO_2 of this urine compared with that of the uncentrifuged sample.

Estimation of total CO_2 - This method was basically the manometric method described by Peters & Van Slyke (1932) but was modified to obtain increased accuracy at the high total CO_2 concentrations found in bovine urine. During early experiments using a 0.2 ml Van Slyke pipette, the repeatability of results from samples containing a high bicarbonate concentration was poorer than the accuracy claimed for the manometric apparatus. After examining many possible sources of error, the 0.2 ml. Van Slyke pipette was replaced by a 0.5 ml. capacity 'Agla' micrometer syringe capable of delivering to an accuracy of .0002 ml. This modification eliminated previous inaccuracies, and repeatability of $\pm$ 0.5% was obtained using a standard bicarbonate solution. In routine analysis, repeatability of $\pm$ 1% was accepted. All samples were estimated in duplicate, and if repeatability was poorer than $\pm$ 1.0% a third estimation was carried out.

Reagents

0.1 N - HCl (boiled and stored under soda-lime)

5.0 N - NaOH

Water (de-ionised, boiled, and stored under soda lime)

Caprylic alcohol

Total CO_2 estimations were carried out on the manometric apparatus of Van Slyke (Peters & Van Slyke 1932). The operator was familiar with the routine handling of this apparatus and conversant with the instructions of Van Slyke regarding its maintenance and operation.

The manometric chamber and cup were cleaned with 0.1 N - HCl and the cup dried with clean gauze. The drops of caprylic alcohol were run into the cup, followed by 2 ml. of water. Exactly 0.2 ml. of the urine sample was then run in from the micrometer syringe by immersing the tip of the syringe below the surface of the water in the cup. This procedure was accomplished without agitation of the fluid in the cup, and, due to its greater specific gravity, the urine could be seen at the bottom of the cup forming a distinct layer below the surface of the water. Urine and water were immediately admitted to the manometric chamber, and a further 1 ml. of water washed into the

chamber. 3.8 ml. of 0.1 N - HCl was then run into the cup, and admitted to the chamber, thus making up a total of 7.0 ml. of fluid from the sample and reagents. The tap of the manometric chamber was then sealed with mercury, the fluid level in the chamber lowered to the 50 ml. mark, and the contents shaken under vacuum for 3 min. The fluid level was then raised to the 2 ml. mark in the chamber and the mercury level in the manometer read to the nearest 0.1 mm. (p1) by an illuminated scale reader, the temperature of the water jacket being noted. 0.3 ml. of 5.0 N - NaOH was then run into the cup, admitted to the manometric chamber, and a mercury seal again applied to the tap. The fluid level was then lowered below the 2 ml. mark, then raised slowly to this mark and the manometric pressure (p2) read. The correction factor (c) was found as described by Van Slyke (1932).

Calculation of the total CO_2 concentration from the pressure readings obtained were made using the conversion factors published for this method.

Estimation of pH In the early part of the investigation pH values in urine were determined electrometrically at room temperature and a conversion factor (Neason, 1953) applied to obtain the correct pH value at 39 C. This method was not ideal due to the

variation in the temperature-pH coefficient at different pH values and different urinary ionic concentrations. Later it became necessary to measure arterial blood pH at body temperature, and this technique was readily usable for measurement of urinary pH. Though great accuracy in urinary pH measurement is possibly not justifiable in routine examinations, it was adopted in this investigation in an attempt to improve the methods on which previous data had been based, and also because the slight differences of pH cause significant errors in calculations using the Henderson-Hasselbach formula.

(1) pH Measurement at Room Temperature. Electrometric pH measurement was made on a Direct Reading pH Meter*. The electrode system was a reference calomel electrode and a syringe glass electrode. When in use, the tips of both electrodes were immersed in a saturated solution of KCl at room temperature.

The meter was set at the beginning of each experiment with freshly prepared buffers+. As the pH of samples was usually between 7.0 and 8.0, buffers at pH $6.99^{(25^{o}C)}$ and at $9.15^{(25^{o}C)}$ were used at the

* Electronic Instruments Ltd. Model 23A.

\+ Buffer Solution Tablets, Burroughs Wellcome & Co.

beginning of each series of measurements to standardise the instrument. Occasionally the scale length was checked against a buffer of pH 4.01 (25°C) Two or more measurements were always made on each sample and the meter was read to an accuracy of 0.02 pH units. The accuracy claimed for this instrument is ± 0.02 pH unit and if duplicate determinations did not agree to within ± 0.02 pH unit, a third measurement was made.

Urine was drawn from below the layer of oil into the syringe electrode with the aid of a record fitting adapter and six inch 16 G Record needle. The adapter was fitted to the syringe nozzle, and urine withdrawn by dipping the needle through the layer of oil into the urine. The glass electrode was washed three times with urine before taking the sample for measurement. Having expelled all air bubbles and ensured the absence of any globules of liquid paraffin, the sample was drawn in by withdrawing the plunger about one inch in the barrel and fitting a rubber spacer round the neck of the plunger to prevent its weight expelling the sample when in the vertical position. The electrode was then clipped to the stand and the electrode assembly lowered into the saturated KCl solution. The reading was recorded after two minutes, or when the meter was stable. The syringe electrode was washed

with distilled water at room temperature between different samples, but was not washed between duplicate determinations on the same sample. To obtain the pH value at 39°C a correction of 0.0053 pH unit per degree Centigrade was made (Weeson, 1953).

(ii) pH Measurement at 39°C A Vibron Electrometer* with a pH Measuring Unit+ and an E.I.L. - Mendel electrode assembly were used in this method. The electrode assembly was basically a reference calomel and a syringe glass electrode connected by a KCl bridge and immersed in a water-bath of constant temperature.

Initially, this system was unsatisfactory due to the occurrence of marked drift in the meter readings. This was thought to result from cooling the electrode system when it was slipped out of the water-bath in changing samples, and the technique suggested by Wilson, (1951) was adopted to reduce this source of error. The electrode assembly and water-bath were placed inside an incubator maintained at a constant temperature of about 36°C. All manipulations were carried out through rubber arm-holes in the Perspex inner door of the incubator (Fig. 1.) This modification resulted

* Model 33D, Electronic Instruments Ltd.
+ Model C - 33 - B, Electronic Instruments Ltd.

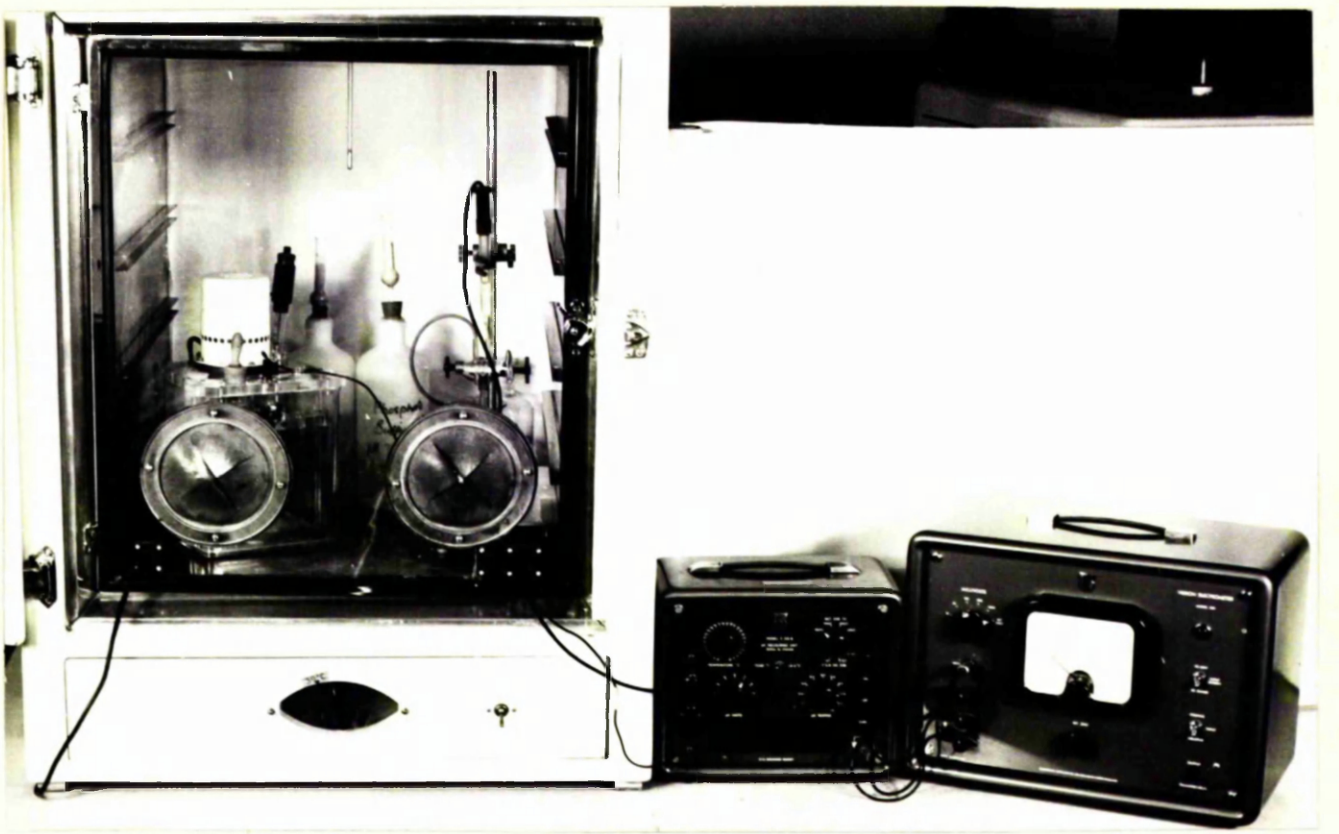


Fig. 1. Apparatus used for the electrometric measurement of blood and urine pH at 38°C. The E.I.L. - Mendel electrode system and controlled temperature water bath are housed with buffers in the incubator with rubber arm-holes. The E.I.L. Vibron Electrometer and pH attachment are shown on the right.

This modification resulted in almost complete elimination of drift. Setting of this instrument was carried out on buffers prepared as described by Bates & Acree, (1945), and Hamer, Pinching & Acree, (1946), and Manov, DeLollis, Lindvall & Acree, (1946) on the recommendation of Mattock, (1959). Three buffers were prepared, an 0.05 molal potassium hydrogen pathalate (pH = 4.027 at 39°C),-a mixed phosphate buffer of 0.025 molal disodium hydrogen phosphate and 0.025 molal potassium di-hydrogen phosphate (pH = 6.838 at 39°C)- and used to standardise the instrument initially. For daily use the meter was standardised on the phosphate buffer only, as suggested by Mattock. The repeatability claimed for the Vibron electrometer and pH attachment is ± 0.002 pH unit. Using the E.I.L. - Mondol electrode system at 39°C, it was not possible to obtain repeatability better than that shown on Table 1. This standard is, however, considered acceptable by most workers (Mattock, 1959).

It was found that in changing from reading one buffer to another widely different in pH value, there was invariably a delay in attaining the new pH. This delay could be minimised by repeated washings with water or saline between buffers. When, however, the theoretical values of the buffers were not widely

Table 1

Results of pH measurement at 39°C. of four phosphate buffers[x] using 0.05 molal potassium hydrogen phthalate as a primary standard.

No. of results	Mean pH(39°C)	Range	S.D.
12	7.252	7.244 - 7.262	± 0.005
12	7.352	7.340 - 7.361	± 0.008
12	7.460	7.453 - 7.468	± 0.005
20	7.571	7.563 - 7.575	± 0.002

[x] Buffer Solutions, British Drug Houses Ltd. Electrometrically checked pH values ± 0.01 pH unit at 20°C.

separated, this delay was greatly reduced. This phenomenon was attributed to the construction of the syringe glass electrode in that it was difficult to wash away completely the ions of the previous buffers from the small interspace between plunger and barrel. By using a buffer close to the expected range of urine pH, and by repeated (3 times) washing with water, any error resulting from the delayed attainment of a new pH value was minimised.

Having checked the instrument on a phosphate buffer, the glass electrode was washed and filled with urine as described for pH measurements at room temperature. The electrode was then returned to the assembly in the water-bath and the pH reading at maximum sensitivity taken at 3 minutes. Measurements were made at least twice and were considered acceptable if within $\pm$ 0.005 pH unit of each other.

RESULTS

The effect of exposure to air on the total CO_2 concentration of urine samples is shown on Table 2. The samples were collected in pairs of tubes as described, one sample of each pair being collected under oil, and the other exposed to air. There was a fall in total CO_2 concentration of all samples

Table 2

Percentage fall in total CO_2 concentrations of bovine urine samples when exposed to air.

Sample No.	Total CO_2 concentration		CO_2 loss %
	Under oil (mM/l)	Exposed to air (mM/l)	
1	250.6	226.1	10
2	223.9	202.4	10
3	90.5	65.1	28
4	8.6	6.6	23
5	5.4	1.5	72
6	3.4	0.8	65

exposed to air when compared with the samples collected under oil. The percentage drop was much greater in the samples with low initial concentrations than in those containing a high total CO_2 concentration.

Pairs of the above tubes were stoppered and left standing at room temperature for about 15 hours, and the total CO_2 after this period compared with that measured immediately after sampling. Table 3 shows the loss of CO_2 which occurred in this time.

There was little loss of CO_2 from the samples containing a high initial concentration when covered with liquid paraffin. The same samples showed a much greater loss when there was a urine-air interface, despite the fact that the tubes were stoppered. A much larger percentage loss occurred from the samples of low total CO_2 concentration and the loss from the urine-air interface was greater than that from the urine-oil interface.

The effect of centrifugation on the total CO_2 concentration of three samples is shown in Table 4. There was a fall in the total CO_2 concentration of the samples which were centrifuged, despite the fact that the tubes were stoppered and the samples were under oil. While the percentage fall at the high total CO_2 concentration was negligible, that at the low

Table 3

Percentage fall in total CO_2 of stoppered urine samples with a urine-oil interface, and a urine-air interface 15 hrs. after sampling.

Sample	Total CO_2 concentration		CO_2 loss %
	2 hr. samples (mM/l)	15 hr. samples (mM/l)	
1. Urine-oil	166.7	163.9	2
1. Urine-air	164.3	154.4	6
2. Urine-oil	159.8	157.9	1
2. Urine-air	156.4	150.2	4
3. Urine-oil	90.3	88.0	3
3. Urine-air	89.5	78.4	12
4. Urine-oil	75.8	75.3	1
4. Urine-air	75.2	70.2	7
5. Urine-oil	10.3	9.8	5
5. Urine-air	9.7	8.5	12
6. Urine-oil	3.4	2.9	15
6. Urine-air	0.8	0.3	63

Table 4

Percentage fall in total CO_2 concentration of bovine urine samples collected under oil, stoppered, and centrifuged.

Sample	Total CO_2 concentration		CO_2 loss %
	Centrifuged (mM/l)	Uncentrifuged (mM/l)	
1	202.6	200.9	1
2	44.7	43.6	2
3	3.3	2.9	12

concentration was, as in the previous experiment, quite marked. The cloudiness in these samples was not removed by centrifugation, but remained suspended in the sample, but slightly less dispersed. Its appearance and behaviour suggested that it was mucus of a similar specific gravity to urine.

Table 5 shows the pH values found in urine samples collected under liquid paraffin and stoppered. In seven of the eight samples the pH after 4 hours was higher than the pH after 1 hour. The mean pH rise in these samples was 0.012 pH unit. In sample 6 the pH fell during this period by 0.016 pH unit. The mean pH change of the 8 samples was 0.013 pH unit.

The pH values of the samples collected without precautions to prevent loss of CO_2 are shown in Table 6. In all eight samples the pH after 4 hours was higher than the pH after 1 hour. The mean rise was 0.080 pH unit. This is a significantly greater rise in pH than occurred in the samples under oil. ($p < 0.01$).

Comparison of Tables 5 and 6 shows that the samples exposed to air for 4 hours had an average pH value 0.098 unit higher than those collected under oil and measured within 1 hour after collection.

Table 5

Change in pH which occurred between 1 hr. and 4 hrs. after sampling in urine samples under oil and stoppered.

Sample	pH		pH
	1 hr	4 hr	
1	7.787	7.808	+ 0.021
2	7.873	7.879	+ 0.006
3	7.227	7.249	+ 0.022
4	7.627	7.639	+ 0.012
5	6.584	6.589	+ 0.005
6	7.255	7.239	- 0.016
7	6.395	6.400	+ 0.005
8	7.626	7.643	+ 0.017
			Mean 0.013

Table 6

Change in pH which occurred between 1 hour and 4 hours after sampling in urine samples exposed to air.

Sample	pH		ΔpH
	1 hr	4 hr	
1	7.824	7.891	+ 0.067
2	7.883	7.949	+ 0.066
3	7.243	7.354	+ 0.111
4	7.655	7.738	+ 0.083
5	6.590	6.690	+ 0.100
6	7.292	7.368	+ 0.076
7	6.368	6.436	+ 0.068
8	7.669	7.734	+ 0.065
		Mean	+ 0.080

DISCUSSION

There was a loss of CO_2 from all urine samples exposed to air. Though the percentage fall in total CO_2 was greatest in those samples with a low initial total CO_2, the absolute amount lost was greatest in the samples with the high initial total CO_2 concentrations. In urine with a high total CO_2 concentration it will be shown (p.142) that there is usually a high pCO_2 and hence a steeper CO_2 concentration gradient between urine and air, thus accounting for a greater tendency for CO_2 to leave the urine sample. If it is accepted that urinary carbonic acid concentration is rarely less than that of blood (1.4 mM/l.), then the large percentage loss of total CO_2 in samples of low concentration is to be expected. At low total CO_2 values, say 5 mM/l, the volatile fraction (carbonic acid) contributes nearly 50% of the total CO_2, thus loss or severe reduction of this fraction by exposure to air will cause a large percentage fall in the total CO_2 concentration.

Comparison of total CO_2 concentrations after 15 hours at room temperature with initial concentrations showed that there was a further fall in concentration over this period of time.

Centrifugation of urine samples in sealed tubes,

and under oil, resulted in a fall in total CO_2 concentration of urine. CO_2 was probably lost to the mineral oil as a result of agitation of the sample in centrifugation. Mineral oil merely retards, but does not prevent, the escape of CO_2 from urine, this effect being due to the slowing down of the CO_2 molecules at the urine-oil interface, rather than the insolubility of CO_2 in oil. CO_2 is in fact rather more soluble in oil than in water (Kubie, 1927).

There was an immediate and continuing elevation in the pH of bovine urine samples exposed to air. This change was negligible when the samples were stoppered and kept under oil. Marshall, (1922) compared human urine samples collected and stored without exposure to air to samples of 5-10 ml. shaken for 1 min. in a 250 ml. Pyrex flask. He found that after 1 min. shaking, the pH of alkaline urine might be elevated by as much as 1.15 pH units, and stated that these conditions might be taken to represent the treatment of samples when no precautions were taken. While the elevation in pH found in the present work was much less than that found by Marshall, the bovine urine samples exposed to air in the present work were not subjected to any agitation.

It was concluded that it is necessary to take

careful precautions to prevent loss of CO_2 when measuring the total CO_2 concentration, and pH of bovine urine samples. Any data obtained from methods which do not include these precautions is likely to show erroneously low total CO_2 concentrations and erroneously high pH values. Anaerobic collection of urine samples as described preserves urinary pH almost unchanged for 4 hours at room temperature.

SUMMARY

Several experiments were carried out to show the effect of exposure to air on the total CO_2 concentration and pH of bovine urine. Total CO_2 concentration fell, and pH rose on exposure of samples to atmospheric air. Collection and storage under oil in stoppered tubes minimised these changes. It was concluded that precautions to prevent loss of CO_2 were essential in all experiments depending on accurate measurement of these urinary constituents in the cow.

Section 2

Measurement of urine flow in cows

Measurement of urine flow in cows

It has been pointed out (Smith, 1951; Anderson & Pickering, 1961) that the accuracy of results in short term investigations of renal function is largely determined by the efficiency of the urine collection technique. Various factors pertaining to accurate urine collection have, therefore, been examined. This work has been described under the following headings:-

(a) Effect of stress during urine flow measurements

(b) Some observations on the use of regional, epidural, and topical anaesthesia during urine collection by an indwelling catheter

(c) Intracystic pressure in the cow

(d) Urinary 'delay time.'

(e) The technique and accuracy of urine collection

(a) Effect of stress during urine flow measurements

In the initial studies of bicarbonate clearance in cows, it was noticed that some animals showed a marked increase in urine flow when puncture of the brachial artery was carried out. As a result of this observation, experiments were carried out to demonstrate the quantitative effect of the painful stimulus of arterial puncture on the rate of urine flow.

METHODS

The animals used were non-lactating, non-pregnant Ayrshire dairy cows accustomed to experimental procedures, and the stimulus was puncture of the brachial artery by the technique described by Fisher (1956). He advocated the use of subcutaneous infiltration anaesthesia to minimise the discomfort of this procedure, but despite its use, discomfort was always evident on penetration of the deeper tissues. The brachial artery is closely related to the first rib (Sisson & Grossman, 1953) which is very sensitive to touch by hypodermic needle.

Arterial puncture was carried out during continuous urine collection by an indwelling urethral catheter.

In order to show that the apparent increase in urine flow was a true diuretic response rather than the expulsion of uncollected urine from the bladder, the colour of urine samples, urinary bicarbonate concentration, and the intra-cystic pressure were measured over the period of arterial puncture. Optical density of urine samples was measured in the E.E.L. colorimeter, total CO_2 concentration was measured as described (p.24.) and intracystic pressure was measured as described in section 2(c).

RESULTS

The response of urine flow rate to the stimulus of arterial puncture in six experiments on 6 cows is shown in Fig. 2.

Fig. 2(a) shows a rise in rate of urine flow in the 20 mins preceding arterial puncture. This was related in time to the preparatory procedure leading up to puncture. These preparations entailed clipping the site near the point of the shoulder, swabbing with antiseptic, and subcutaneous infiltration with local anaesthetic. The stimulus of puncture resulted in a sharper rise in rate of flow, followed immediately by a steep fall back to preliminary measurements.

This general pattern was repeated in the other

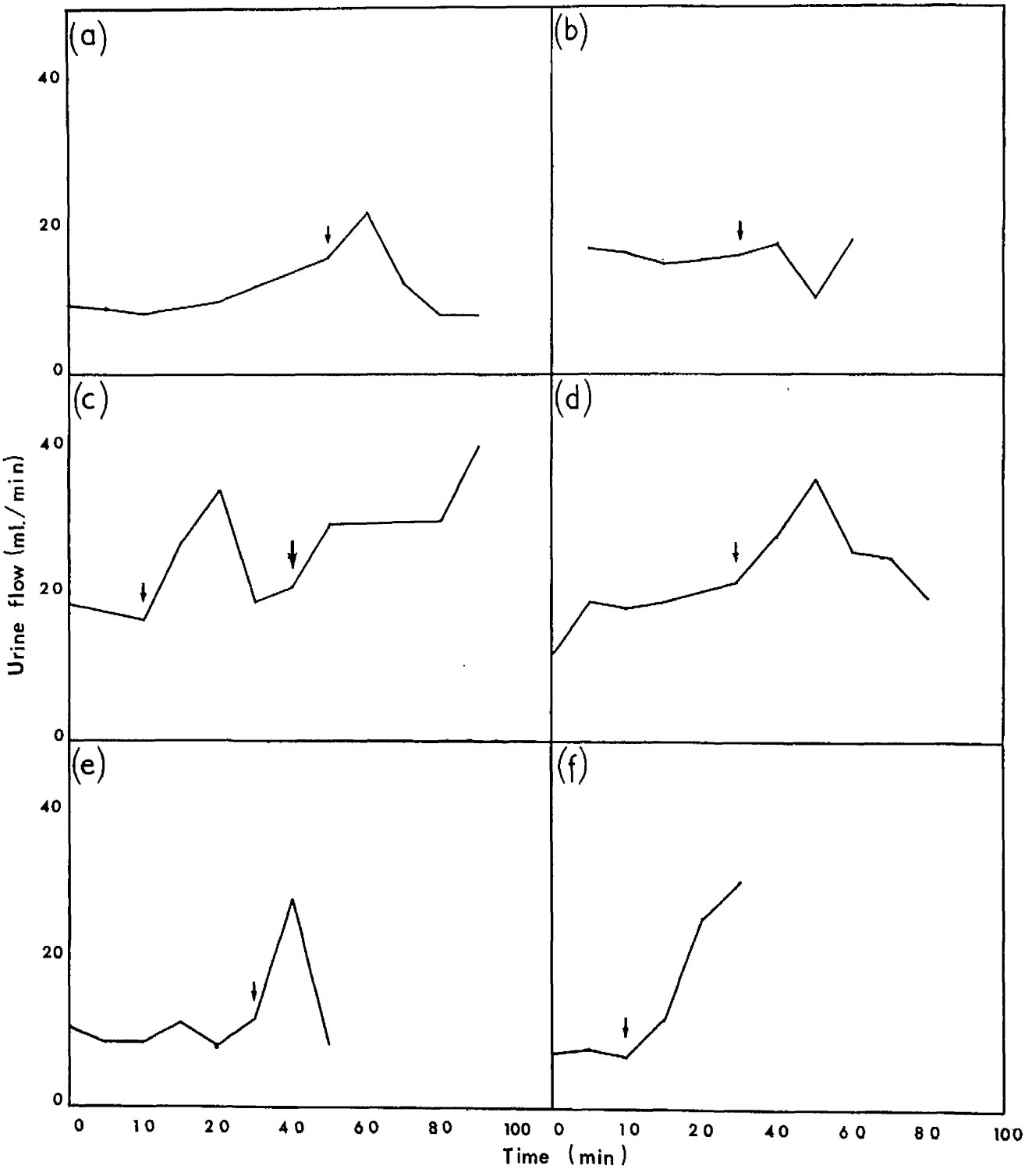


Fig. 2. The effect of arterial puncture on urine flow rate in 6 experiments on 6 cows. The arrow indicates the time of arterial puncture. In (c), the first arrow indicates preparation of the site for arterial puncture, and the second indicates the execution of arterial puncture.

observations except in Fig. 2(b) where there was only a slight rise followed by a sharp fall and subsequent rise in the rate of urine flow following arterial puncture. In 2(c) the time of preparation of the site was noted exactly, and fortuitously, there was a more marked response on this occasion than on any other. These results were selected because with the exception of 2(b), they showed a fairly clear response to the stimulus of arterial puncture. On other occasions, the response was ill defined, or non-existent. On no occasion was a clear antidiuretic response noted.

Fig. 3 shows the relationship between changes in the rate of flow, optical density, bicarbonate concentration and bicarbonate excretion resulting from arterial puncture in one experiment. It is clear that despite a fall in total CO_2 concentration in response to the diuresis, the rate of bicarbonate excretion was enhanced. Urine pH was not measured in this experiment, but the relationship between pH and total CO_2 concentration in urine is such that it is certain that a fall in pH accompanied the diuresis. A fall in pH was noted during diuresis in other experiments.

DISCUSSION

The influence of pain or emotional stress on the

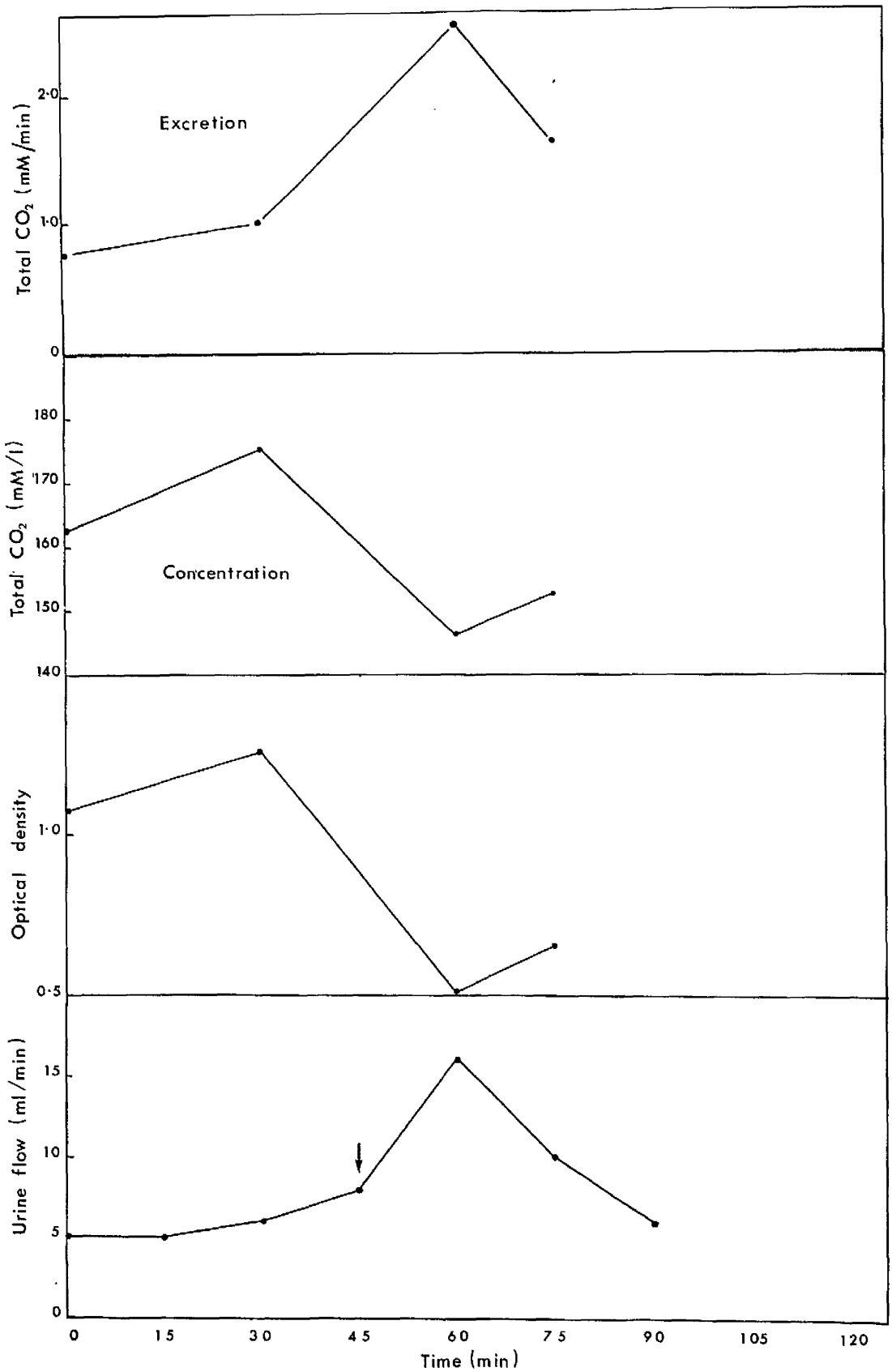


Fig. 3. Variations in urine flow, optical density, and bicarbonate concentration and excretion following arterial puncture. The arrow in the lowest graph indicates the time of arterial puncture.

rate of urine flow in animals and man has attracted the attention of various workers. Rydin & Verney (1938) demonstrated a marked inhibition of urine flow of hydrated dogs following emotional stress, with good evidence that this was due to the release of anti-diuretic hormone. Emotional disturbance, and painful stimuli in the rabbit caused antidiuresis which was related to decrease in the renal blood flow (P.A.H. clearance) and filtration rate (creatinine clearance) according to Brod & Sirota (1949). Andersson & Persson (1958), however, reported that in hydrated goats, painful or emotional stimuli did not result in any alteration in the rate of urine flow. In women, Miles & de Wardener (1953) described an emotional diuresis which occurred on bladder catheterization, or resulted from apprehension of a surgical procedure. They attributed the rise in salt excretion which accompanied this diuresis to three possible causes: (1) a rise in glomerular filtration rate; (2) the inhibition of salt-retaining hormone; (3) a nervous inhibition of tubular function. There are, therefore, marked species differences in renal responses to painful or emotional stimuli.

The importance of mental or physical stress in renal clearance studies in the cow has been emphasised

by Knudson (1960). His animals showed mild to severe excitement during experimentation. A 43% fall in glomerular filtration rate was attributed to 'excitement corresponding to hard muscular work' in one cow, while he concluded that '.....it was a mental stress to the cow to be involved in clearance experiments.....' and that 'the excitement.....seemed.....to greatly affect electrolyte clearances.'

There is some evidence to support the belief that the rise in urine flow found by the above workers, and by the author, is at least in part related to alterations in renal haemodynamics. Goodwin, Harris & Scott (1950) found that following stimuli to hind limbs, there was a decrease in 'renal function' during and after stimulation, which was often followed by an increase, thought to represent reactive hyperaemia. According to Doyle, Patterson, Warren & Detweiler (1960), mean blood pressure in cows may rise markedly on experimental interference, so it is probable that the rise in urine flow resulting from a painful procedure to cattle which is reported here, was in part related to alterations in renal haemodynamics.

An alternative or perhaps contributory cause of the diureses observed was hyperpnoea. A noticeable effect of the procedures described in this work was

that apprehension of pain, and pain itself caused rapid blowing respiration by experimental animals. Currie & Ullman (1955) showed that voluntary over-breathing without attendant hypo- or hypercapnia can cause a marked enhancement of water excretion. No increase in electrolyte excretion accompanied this polyuria. Kanter (1961), however, found that no change occurred in the glomerular filtration rate, renal plasma flow, or filtration fraction when anaesthetised dogs were hyperventilated.

Measurements of total CO_2 concentration and colour of the urine showed that a marked dilution of urinary pigment, and total CO_2 occurred during diuresis. This discounted any likelihood of the apparent diureses being due to expulsion of uncollected urine from the bladder. Intracystic pressure did not alter during diuresis. As a result of the marked increase in urine flow, renal bicarbonate excretion was increased, though the concentration fell. This is in accord with the findings of Nutbourne & de Wardener, (1960) who showed that the rise in urinary pH which followed a water diuresis in man (excreting an acid urine) was not simply a dilution effect but was due to a fall in the secretion of hydrogen ions. In man, no measurable effect on bicarbonate excretion could be shown as it

is totally reabsorbed, but in the cow any impairment in hydrogen ion secretion results in a marked increase in the rate of excretion of bicarbonate as shall be shown.

The results of these experiments had a bearing on the course of the programme of measurement of bicarbonate clearance in the cow. In order to obtain accurate measurements of the renal clearance of a substance, blood must be sampled in the course of urine collection. It is clearly undesirable that marked fluctuations in urine flow such as are reported here, should occur during the collection period. The technique of collecting arterial blood (Fisher, 1956) was therefore deemed unsuitable for use in the measurement of bicarbonate clearances in cattle. An alternative and, from the point of view of clearance studies, more convenient technique for obtaining arterial blood from the adult dairy cow is described elsewhere.

Painful stimuli other than that described above may have a similar effect on diuresis. It has been observed in routine clearance studies that when the discomfort of a urethral catheter increases as the effect of epidural anaesthesia wears off, a sharp rise in urine flow frequently occurs. This response is abolished on re-induction of satisfactory epidural anaesthesia. It is, therefore, important that in

renal function studies in the conscious cow procedures causing discomfort be avoided, and that where possible, local or regional anaesthesia be used to abolish pain.

SUMMARY

The diuretic response to a painful stimulus in 6 experiments on 6 cows was described.

The results were discussed in relation to the findings of other workers.

It was concluded that any technique resulting in pain or discomfort may cause marked diuresis in the cow and is, therefore, undesirable in renal function studies.

(b) Some observations on the use of regional epidural and topical anaesthesia during urine collections by an indwelling catheter.

It is important in physiological investigation that the physical and psychical disturbance to the experimental subject be minimal. In the conscious subject, the use of local and regional anaesthesia must be considered where any manipulative or minor surgical procedure is contemplated.

Several techniques for collection of urine from cattle have been described, but in none has the use of any form of anaesthesia been advocated.

Cunningham, Frederick & Brisson (1955), using a self-retaining rubber catheter with an inflatable bulb reported 'some discomfort' in cows when first catheterised. Other workers (Poulsen, 1957; Sellars, Pritchard, Webber & Sautter, 1958; Knudsen, 1960) using various forms of urethral catheters without anaesthesia did not mention evidence of discomfort, but Poulsen (1959) stimulated emptying of the bladder at the end of a clearance period by pulling on the catheter. This resulted in contraction of bladder and abdominal muscles and emptying of the urine into the collecting vessel. The presence of sensation was utilised in this work to

improve the efficiency of urine collection. Knudsen (1960) emphasised the excitement and struggling which occurred during clearance experiments using this technique, and concluded that the abnormal conditions caused such marked variations in electrolyte excretion that 'even the figures from the first (control) periods must be accepted with some reservation.' In the light of Knudsen's and the author's (1961) experience in clearance measurements in cattle, the reduction of discomfort and fear during the present work was given priority. As the main cause of discomfort during clearance experiments is the presence of a urethral catheter or other drainage equipment, the effect of local or regional anaesthesia in the abolition of this discomfort was examined.

(1) Catheterisation without anaesthesia On the basis of the published work, the establishment of continuous bladder drainage by urethral catheter was attempted without anaesthesia. This procedure had several disadvantages.

Insertion of the catheter through the urethra caused marked resentment. When the catheter was passing through the urethra, arching of the back and micturition frequently occurred. Agitated movements of the subject increased the risk of injury to the

urethra and adjacent tissue by the tip of the catheter and stilette.

When in position, the presence of the catheter and inflated rubber cuff resting against the internal urethral meatus caused marked agitation. This varied between individuals, and ranged from slight 'paddling' of the hind feet, tail twitching, and lowing, to repeated or continuous back arching, straining to micturate, and bellowing. Frequent defaecation usually accompanied this agitation. Attempts at micturition and defaecation usually resulted in some loss of urine round the catheter, and occasionally in forceful expulsion of the catheter. At best, the urine flow through the catheter was intermittent.

Apart from the practical disadvantages described, this procedure resulted in a state of distress to the experimental subject which was considered ethically, aesthetically and physiologically undesirable.

There is no doubt that experimental urine collection in cattle without anaesthesia can be carried out with useful results. Where urine collection is made over long periods, the effect of inaccuracies is reduced, and the cattle become accustomed to the presence of the catheter (Cunningham, Frederick & Brisson, 1955). For short term clearance studies,

however, in cattle not necessarily accustomed to catheterisation, the disadvantages described create unsuitable conditions for physiological studies, and consequently their removal by anaesthesia is desirable.

(ii) Catheterisation with anaesthesia. Two types of anaesthesia were used;

(a) local anaesthesia by topical application of amethocaine hydrochloride* to the mucous membrane of the vulva and vagina,

(b) regional anaesthesia by the epidural injection of 5% procaine hydrochloride with adrenaline.+

(a) Topical Anaesthesia

Amethocaine hydrochloride is recommended for analgesia by topical application to mucous surfaces (Wright & Hall, 1961). It was decided to use this method of anaesthesia for two reasons; to ascertain whether topical anaesthesia was a useful alternative to epidural anaesthesia, and, by comparison with epidural anaesthesia, to examine the effect of regional anaesthesia on bladder function.

After thoroughly cleansing the perineal area,

* 'Amethocaine' - Glaxo.

+ 'Planocaine' - May and Baker.

the amethocaine hydrochloride was smeared over the vulva, vagina, external urethral orifice, and urethra. The catheter was introduced into the bladder after allowing 10 to 15 minutes for the development of anaesthesia. Application of amethocaine hydrochloride to the vagina and urethra produced anaesthesia, which, at its best was as effective as epidural anaesthesia for purposes of inserting an indwelling urethral catheter. Signs of discomfort due to the catheter's presence in the urethra were abolished for up to 2 hours.

On several occasions, however, anaesthesia was poor, as evidenced by marked discomfort on introduction of the catheter, and strenuous attempts to micturate while the catheter was *in situ*.

On one occasion, after application of the anaesthetic, but before introduction of the catheter, urine dripped continuously from the vulva. This was thought to be urinary incontinence resulting from application of the topical anaesthetic to the urethra.

Repeated use of the anaesthetic appeared to have no harmful effect on the vaginal mucous membrane.

On no occasion was defaecation reduced or abolished by the anaesthetic.

Traces of the anaesthetic carried into the bladder

at catheterisation, frequently appeared in the urine collected.

These observations indicated that this type of anaesthesia was unsuitable for use in clearance experiments because of its unreliability in producing satisfactory anaesthesia, its failure to abolish defaecation, and the risk of its contaminating urine samples.

(b) Epidural Anaesthesia

Epidural anaesthesia in the cow has been widely used for many years. In reviewing the technique, Brook (1935) credits Benesch (1926) with the first description of epidural anaesthesia in the cow for veterinary clinical work.

The anatomical disposition of the neural canal, nerves, and spinal membranes of the ox make epidural anaesthesia a convenient and rather safe method of abolishing sensation in the perineal area. The spinal membranes terminate about the mid-sacral region. Behind this level at the first and second inter-coccygeal space, therefore, the epidural space occupies the entire spinal canal, and contains only the nerve fibres running to the tail; The space is not completely closed and injected fluid may escape at the intervertebral foramina. The canal terminates

in the region of the fourth coccygeal segment (Brook, 1935).

In selecting a suitable technique for epidural anaesthesia in renal clearance work, the following effects were sought - abolition of sensation from vagina, urethra, and bladder, prevention of reflex micturition and defaecation, and freedom from interference with the motor function of the hind limbs.

Abolition of sensation. It is not clear from the literature what nerves carry efferent impulses from the urethra and vagina of the cow. Wright & Hall (1961) stated that the vagina is innervated by a sympathetic supply via the hypogastric nerves from the posterior mesenteric plexus. The 4th and 5th sacral nerves are sensory to the croup, base of tail, anus, vulva, perineum, and adjacent parts, and are motor to the anus, terminal part of the vagina, penis, bladder and urethra. Prather (1953) stated that sensory nerves, afferent from the bladder and adjacent regions in man are probably present in sympathetic, parasympathetic, and somatic trunks, and may join the spinal cord as high as the ninth thoracic segment. He quoted Langworthy as stating that painful sensations from the dome of the bladder, and sensation of the need to void are carried in parasympathetic trunks to the 2nd and 3rd sacral

segments. Pudendal nerves are stated to convey proprioceptive sensations from the region of the anal sphincter. As a general rule visceral pain fibres follow sympathetic fibres with the exception that fibres from the sigmoid colon and rectum, neck of the bladder, prostate, and cervix of the uterus enter the cord in the dorsal roots of the second to 4th sacral segments, where the parasympathetic efferents originate (Brodal, 1948). It appears that these visceral afferent fibres accompany the parasympathetic nerves in their peripheral course. Pain fibres from the fundus of the bladder pass through the hypogastric plexus to the dorsal roots of the 11th thoracic to the 1st lumbar segments. Garry (1957) drew attention to the fact that there are marked species differences in the innervation of the urinary bladder.

In view of the diffuseness of the afferent nerve supply from the vagina, urethra, and bladder, it was to be anticipated that sensation from these structures would not be completely abolished.

Prevention of micturition and defaecation Despite the fact that it is difficult to anaesthetise the above structures, it is recognised that epidural anaesthesia will abolish the micturition reflex (Wright & Hall, 1961). Both afferent and efferent components of the

nervous reflex responsible for the act of micturition are in parasympathetic pathways. Anaesthesia of the sacral nerve roots will, therefore, abolish micturition. Defaecation is stopped for the same reason.

Interference with motor function of the hind limbs

The lumbo-sacral plexus from which the nerves of the hind limbs are derived receives nerves mainly from the last 3 lumbar and the first 2 sacral nerves (Sisson & Grossman, 1938). If anaesthesia of the hind limbs were to be avoided, therefore, the volume of anaesthetic had to be restricted. Anaesthesia affecting the posterior, but not the 1st and 2nd sacral nerves was required.

In his description of 'posterior epidural' anaesthesia in the cow, Brook (1935) said that the vagina and rectum are insensitive and ballooned, the nerve supply to the bladder and pelvic urethra is blocked, and the motor supply to the hind limbs is unaffected. This technique, therefore, appeared to meet the requirements for catheterisation and bladder drainage.

Technique of epidural anaesthesia

The skin over the sacro-coccygeal space was clipped and swabbed with antiseptic. A No. 0 Hypo (1.5 in. x 20 S.W.G.) needle was then inserted through

the skin into the epidural space as described by Brook (1935). The needle used was shorter and much finer than that recommended by Brook, to minimise the trauma of repeated injections. A 10 ml. syringe containing 4 ml. of 5% procaine hydrochloride was fitted to the needle and the contents injected into the epidural space. The precautions recommended, and the criteria for locating the needle were observed. (Wright & Hall, 1961).

Observations. If the anaesthetic was administered accurately, flaccidity of the tail developed almost immediately, and loss of sensation in the vulva developed during the following five minutes. The catheter was introduced when there was no response to stimulation of the lips of the vulva with a needle or flicking with the fingers.

Micturition, defaecation, and motor function of the tail were usually absent for about 2 hours, and occasionally up to 4 hours. If anaesthesia were off before the urethral catheter was removed, returning sensation was manifested by defaecation, tail twitching, straining to micturate, increased urine flow, and other evidence of discomfort. These signs were readily abolished by reinforcing anaesthesia with a further 3 ml.

of anaesthetic into the epidural space.

Most of the effects described by Brook (1935) and Wright & Hall (1961), were seen to occur in the present work. Some observations were recorded, however, which did not concur with the reports of these authors.

Wright & Hall call in question the effectiveness of 2 to 3 ml of 5 per cent Procaine for producing epidural anaesthesia. In the present work, 4ml of 5 per cent Procaine hydrochloride was found to cause lasting posterior epidural anaesthesia, and in certain circumstances (v.i.), 3ml injected into the sacro-coccygeal space caused not only anaesthesia, but some motor paralysis of the hind limbs.

The increased rate of urine flow which occurred as the anaesthetic were off usually regressed when anaesthesia was reinforced. This diuresis was thought to be associated with the discomfort of the catheter in the urethra. It has been noted by the author (1961) that pain or the anticipation of pain may cause a marked diuresis in cattle. This effect provided further evidence of the usefulness of epidural anaesthesia in clearance studies in the cow. In experiments without anaesthesia, urine flow could be artificially elevated as a result of the discomfort due to the urethral catheter.

Brook (1935) claimed that, with posterior epidural anaesthesia, the vagina and rectum are insensitive, and that the nerve supply to the bladder and pelvic urethra is blocked. Using the method described, the author never observed complete anaesthesia of the vagina and urethra of the cow, despite the fact that there was complete anaesthesia of the tail and perineal area, ballooning of the rectum, relaxation of the anus and vulva, complete inhibition of the micturition and defaecation, and in some cases, a degree of motor paralysis of the hind limbs. There was reduced sensation but never abolition of sensation from the vagina and urethra. Introduction of the catheter or pulling on the catheter always caused discomfort. This sensation was not, however, interpreted as a desire to micturate by the anaesthetised, as it was by the unanaesthetised, animal.

These observations were not surprising in view of the fact that the nerve supply to the vagina and urethra is partly from the posterior mesenteric plexus which is largely supplied by the 2nd, 3rd, 4th and 5th lumbar nerves (Sisson & Grossman, 1938). A small volume of anaesthetic introduced at the level of the 5th sacral nerve could not be expected to affect the roots of the anterior lumbar nerves.

Complications and side-effects On several occasions, cattle with raised tail-heads were used for urine collection experiments. These animals were usually suffering from cystic ovarian disease - a condition which is usually accompanied by slackening of the sacro-sciatic ligaments and elevation of the tail-head (Dawson 1957, 1958). The spinal canal of these animals tended to slope downwards and forwards from the sacro-coccygeal space. On almost every occasion, injection of the usual amount of anaesthetic resulted in marked ataxia of the hind limbs. Even after reduction of the volume injected to 3ml, ataxia usually accompanied anaesthesia of the perineal area.

Several of the experimental animals were subjected to epidural anaesthesia about ten times, and in the course of this work the technique has been used several hundred times without causing any ill effect. Brook (1935) reports that partial or total caudal paralysis may result from epidural anaesthesia. This may result from damage of the coccygeal nerves by a heavy needle, and it appears that by using a fine needle, such risks are very low. After repeated epidural anaesthesia on one animal, induction may become progressively more difficult. Reduction of the size of the epidural space by fibrosis may be a contributory factor to this

finding.

SUMMARY

Observations of the effects of catheterisation of the bladder of the cow with and without anaesthesia are recorded.

The advantages of the use of epidural anaesthesia in renal clearance work are discussed.

It is concluded that;

1. catheterisation without anaesthesia has disadvantages which are undesirable in short-term experiments measuring the rate of excretion of urinary constituents.

2. topical anaesthesia though occasionally effective in abolishing discomfort is unreliable in effect and duration.

3. when used with care, epidural anaesthesia is a safe and efficient way of abolishing discomfort, micturition, and defaecation in renal clearance experiments in cattle.

(c) Intracystic pressure in the cow

Cystometry, measurement of the hydrostatic pressure in the urinary bladder, has been utilised in man and laboratory animals to extend knowledge of the physiological mechanisms controlling bladder filling and micturition (Denny-Brown & Robertson, 1933; Fulton, 1955; Garry, Roberts & Todd, 1959), and in studying the behaviour of the bladder after spinal injury (Prather, 1953). In man, variations in bladder pressure may be effected by alterations in posture (Gould, Hsieh & Tinckler, 1955). In the upright position, bladder pressure was three times higher than in the horizontal position. This rise in pressure was attributed to the weight of the abdominal contents pressing on the bladder. These workers found, however, when the human subject was tilted to the head-down position, the recorded bladder pressure fell below zero. Rose (1944) also mentioned the existence of 'a slightly negative intracystic pressure in spite of extra-cystic intra-abdominal pressure changes.' It is, however, usually accepted that in man, the bladder contents are at a low positive pressure of about 10cm. of water (Fulton, 1955).

While developing the method of urine collection in

the present work, observations were made on the bovine bladder which merited further investigation by cystometry. The observations and the results of experiments using a simple cystometric technique are described below.

It has been mentioned elsewhere (p.78.) that one of the difficulties of maintaining continuous drainage of the bovine bladder results from the presence of air-locks in the catheter and the collection tubing. This air could only gain entry during or after catheterisation, as it is not normally present in the bladder.

During the survey of urine pH and bicarbonate from a herd of dairy cows samples were taken by bladder catheterisation of the unanaesthetised cattle. Frequently the stimulus of catheterisation caused simultaneous micturition, but where micturition did not occur, the urine samples could only be obtained after exerting suction on the collection tubing. If the catheter and collection tubing remained patent during and after catheterisation, there was invariably an inrush of air into the bladder. Urine was never passed down the catheter without a micturition response or the application of negative pressure to the tubing. These observations led to several experiments to measure the apparent negative intracystic pressure in

the bovine bladder. Many of these were carried out during the routine experiments on renal clearance.

METHODS

To show the existence of a negative intracystic pressure, a clamped indwelling catheter was introduced into the bladder of a standing cow under epidural anaesthesia and P.V.C. collection tubing attached to the free end of the catheter. The free end of the collection tubing was fixed to a rigid scale marked in inches and centimetres, with the tip of the tubing projecting about 1 in. beyond the zero of the scale. The scale and the tubing was then clamped vertically so that the tubing was immersed in a beaker of coloured water up to the zero mark of the scale (Fig. 4a). With the beaker at floor level, the clamp was removed from the catheter and the height of the fluid column measured. Having measured the intracystic pressure thus, the bladder was drained and the pressure again measured after displacing the fluid contents of the tubing with air. Thus bladder pressure measurements were made before and after emptying.

As measurement of positive pressures was not possible by the above method, the collection tubing was modified to act as a simple water manometer. An 18 in.

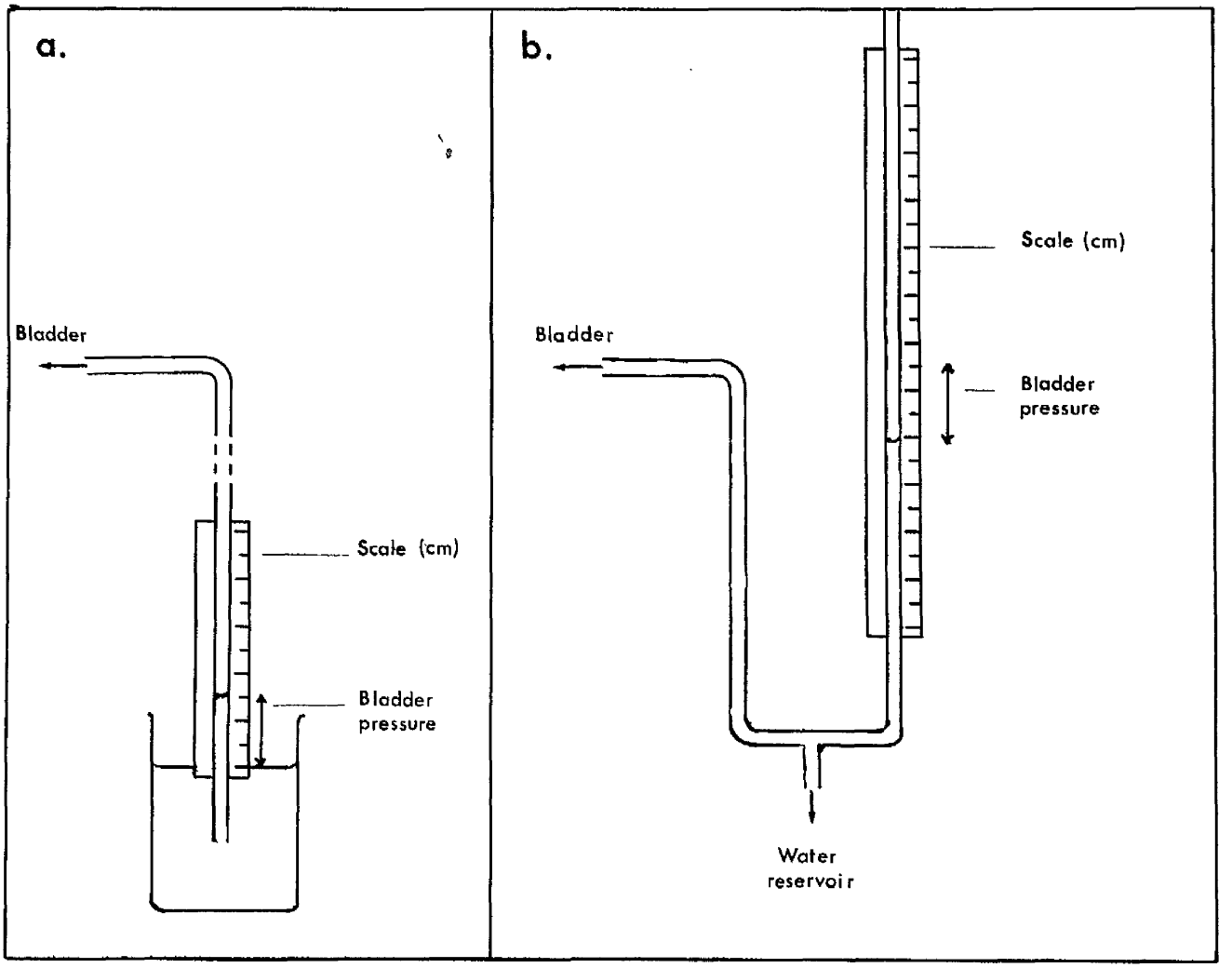


Fig. 4. Apparatus used for the measurement of intracystic pressure in the cow.

(a) 'Beaker' method demonstrating negativity of bladder pressure.

(b) Manometric measurement of intracystic pressure during bladder filling.

length of glass tubing was fitted to the end of the collection tubing and fixed to a scale. The tubing was then filled with urine from the bladder and the manometer set up as shown (Fig. 4b). The relation of the urine level in the tubing to the zero on the scale could be altered by raising or lowering the manometer. The level of the manometer relative to the cow was therefore important to absolute, but not to comparative readings. This level could be fixed from a knowledge of the negative pressure found as described in the previous paragraph. If the 'bladder' method showed a negative pressure of 5cms. of water, then the manometer was fixed to show a reading of -5cms. Thus the position of zero on the scale represented atmospheric pressure and also was approximately level with the top of the cows bladder.

Bladder pressure was measured by this method in 3 cows. Fluctuations in pressure were related to respiration, eructation, coughing, bladder filling, and micturition. A comparison was made in one cow between the manometric pressure recorded during epidural anaesthesia and during topical anaesthesia. In another experiment intra-abdominal pressure was measured at the same time as the cystometry. A large bore needle was introduced through a disinfected area

of skin on the right flank in the sub-lumbar fossa. This was attached by pressure tubing to a manometer.

RESULTS

In five experiments on 3 cows, bladder pressure was measured 5 min after complete emptying of the bladder by catheter. Table 7 shows the results obtained. In each experiment a negative bladder pressure was recorded (range -3.5 to -10.0cm). The volume of urine removed from the bladder was not measured, but it is possible that the variation in the post-drainage pressure in the individual cow may have been related to the state of fulness and stretch of the bladder wall which existed prior to drainage.

Respiration. There was a continual fluctuation in the manometric pressure which was related to inspiration and expiration. On inspiration the pressure increased and on expiration, it decreased. The range of fluctuation was 0.5 to 1.0 cm. Respiration was associated with slight movement in the standing animal so that the fluctuations were partly attributable to movement rather than actual intracystic pressure changes. That respiratory effects were important to bladder pressure was, however, evident from the observations noted in the next sections.

Table 7

Pressure of the bovine bladder 5 min after emptying.

Case No.	Bladder Pressure ($cm\ H_2O$)
9488/1	-6.0
"	-6.5
"	-9.5
213	-7.0
12916	-5.0 to -10.0 [x]

[x] This cow was restless during measurement thus a steady pressure reading was not obtained.

Coughing This had a marked effect on bladder pressure. Bladder pressure was often elevated from -6cm. to + 30cm. Again the spasmodic movement associated with a cough affected the manometer reading, but the pressure increase was much in excess of that noted in other movements not associated with respiratory effort.

Eructation Immediately before eructation, respiration was inhibited, the bladder pressure then rose by about 5cms and eructation occurred. Bladder pressure then returned immediately to its original level.

Micturition When the effect of anaesthesia of the vagina and urethra were off, micturition was usually stimulated by the presence of the urethral catheter. An effort to micturate caused a rise of about 32cms of water in the manometer reading. Micturition was of course accompanied by the characteristic arching of the back and lowering of the vulva, thus the effect of movement and alteration of the height of the bladder relative to the manometer also contributed to the pressure change.

Fig. 5 shows the bladder pressure changes recorded during coughing, eructation, and micturition. Bladder filling did not occur during this experiment due to continuous drainage by the catheter.

Diuresis associated with arterial puncture Diuresis

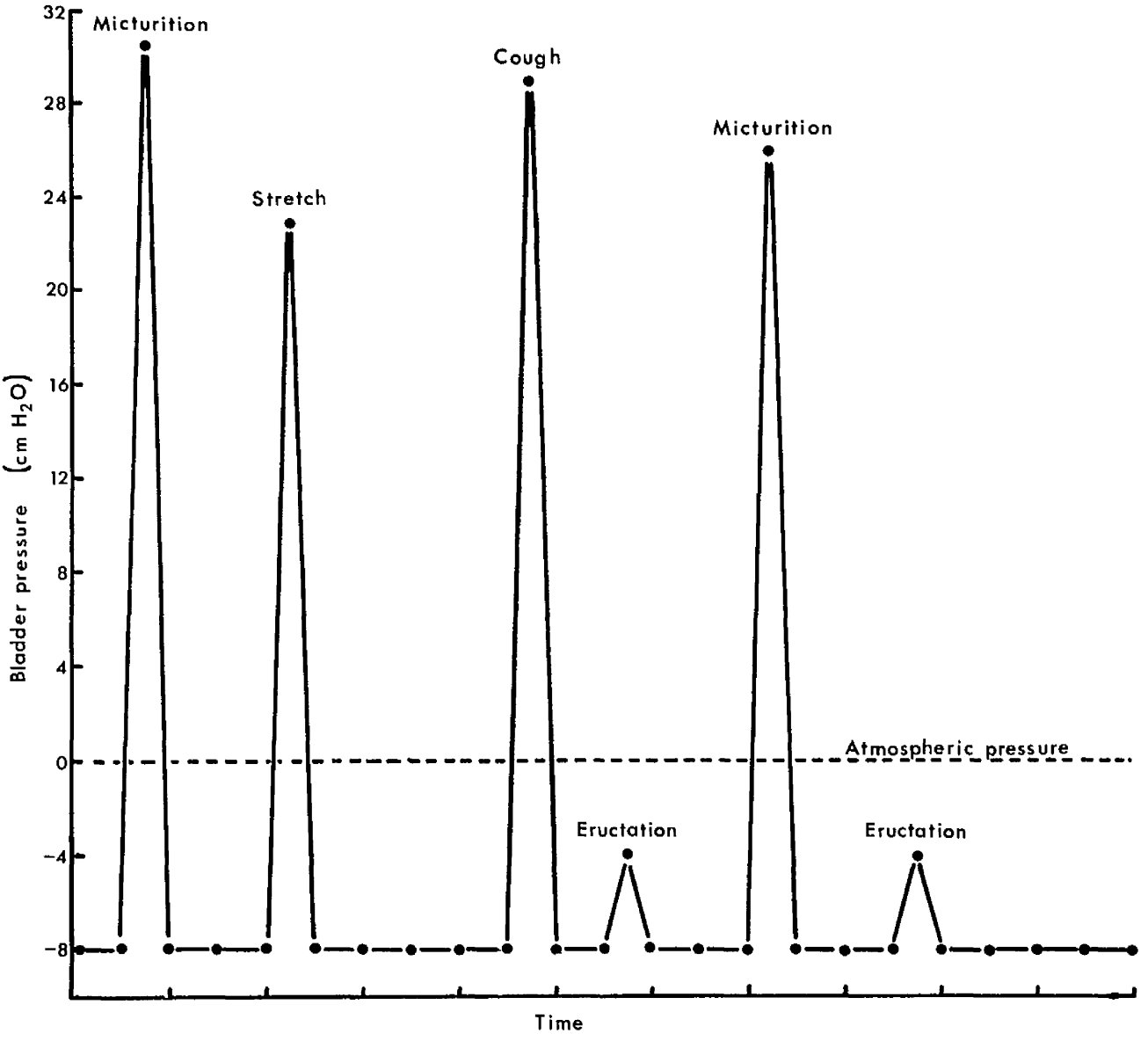


Fig. 5. The changes in bladder pressure due to detrusor contraction and extracystic pressure changes. The first micturition pressure change was before induction of epidural anaesthesia, subsequent changes were recorded under the influence of anaesthesia, and the final micturition response occurred as the anaesthetic was wearing off. For clarity, the small fluctuations in intracystic pressure are not shown (see text). The graph is illustrative of observed changes in bladder pressure and, for convenience, the changes are not shown in the sequence in which they occurred, nor are the time intervals to scale.

occurred in some animals in association with attempts at arterial puncture (Anderson, 1961). To show that this was a true increase in the rate of urine flow, rather than a reflex contraction of the bladder resulting in the expulsion of previously uncollected urine, bladder pressure was measured during the diuresis. No alteration in bladder pressure was noted during the diuresis resulting from arterial puncture.

Epidural anaesthesia In three experiments catheterisation was carried out after applying a topical anaesthetic to the vagina and urethra. The bladder was then drained and the intracystic pressure measured. An epidural anaesthetic was then given and, after 5 - 10 min, the pressure again noted. It was hoped that, as posterior epidural anaesthesia abolishes the detrusor response in the cow (q.v.), the experiment might show whether this effect was associated with alteration of the tonus of the bladder wall, resulting in a change in bladder pressure.

The results of the three experiments were inconclusive. In two experiments there was no alteration in bladder pressure as a result of epidural anaesthesia, but in a third, the negative pressure increased from -4 to -10cms.

Bladder filling A cystometrogram was constructed by

recording the effect of known increments of fluid on bladder pressure. The addition of 2,000 ml of water in 250 ml aliquots resulted in increasing the bladder pressure from -5cm to +11cm of water. In another animal, a 2,500 ml increment resulted in a pressure increase from -9 to +4cms of water. The pressure changes which occurred during these experiments are shown in Fig. 6 . After emptying the bladder of a large volume of fluid (2,500 ml), the pressure fell 3.5cms below the pre-filling pressure, but returned to the pre-filling pressure 8 minutes after emptying.

Introduction of air If the catheter and collection tubing were emptied of fluid, and opened to the atmosphere, there was an immediate inrush of air into the bladder, which was accompanied by 'gurgling' sounds. Despite epidural anaesthesia, the entry of this air into the bladder induced micturition on several occasions - even although a large volume of water had previously failed to induce micturition. Micturition caused the expulsion of much air, and whatever urine remained in the bladder.

Relationship of intracystic and intra-abdominal pressure Intra-abdominal pressure was measured in one experiment at the same time as the intracystic pressure. It was hoped to demonstrate the interdependence of

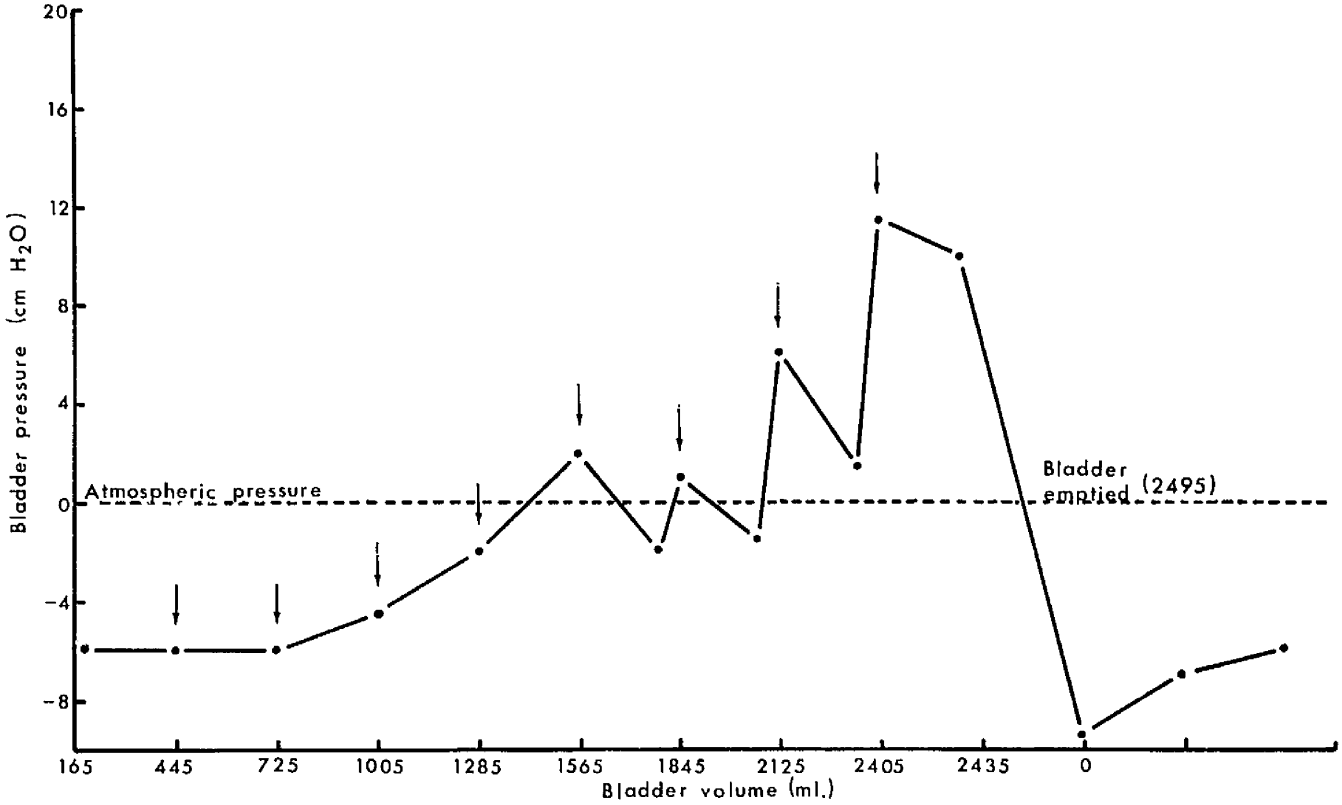


Fig. 6. The effect of filling on intracystic pressure. Case no. 9488/1. 250 ml. of distilled water at 38°C was added at 5 min intervals to the bladder via the catheter. The arrows mark the completion of each 250 ml. infusion. Urine flow before the infusions was 6 ml./min, thus during infusion, the estimated inflow was 6 x 5 + 250 = 280 ml. in 5 min. The estimated volumes in the bladder are shown on the abscissa. 2495 ml. was collected from the bladder at the end of the infusion. The estimated volume was 2000 ml. (infused) + 465 ml. (urine) = 2465 ml.

intracystic and intra-abdominal pressure. Both intra-abdominal and intracystic pressure were negative and approximately equal (-3.5cms). The tubing leading from the abdominal cavity was then opened to the atmosphere for 2 - 3 mins and air was allowed to enter. When the tubing was then closed off the negative pressure was abolished initially, but returned to its previous negative intra-abdominal pressure. No alteration in the intracystic pressure was noted during this procedure.

DISCUSSION

No other reports have been found on the hydrostatic pressure of the bovine bladder. There is evidence that under certain circumstances, a negative intracystic pressure may occur in man; Rose (1944) stated that the bladder musculature may maintain a slightly negative intracystic pressure in spite of extracystic intra-abdominal pressure changes, such as coughing and changes in position. He believed that the bladder pressure was independent 'in a degree' of intra-abdominal pressure, but that it was influenced by sudden extracystic changes, and that contraction of the bladder wall could be stimulated by pressure

changes outside the bladder. This hypothesis may contribute to an explanation of the variability of bladder responses to filling under different circumstances. As it appears that the micturition reflex can be under extracystic as well as intracystic influence, the increased frequency of a desire to micturate under conditions of cold or excitement may result from the increased tonus of the abdominal musculature. Gould et al (1955) examined the effect of posture on bladder pressure in man. They found that change of posture from a horizontal to an upright position caused a three-fold or more rise in bladder pressure. Tilting to a head down position caused a sharp fall in the recorded pressure, and in four subjects who were tilted in this direction to the full 90°, recorded bladder pressure fell below zero. Unfortunately, the authors did not examine the bladder pressure while the subjects were on all fours, but, as posture is so influential in modifying intracystic pressure, it is not surprising that a species of quite different conformation and posture should have a quite different intracystic pressure.

Respiration and all respiratory efforts such as coughing and bellowing result in contraction of diaphragmatic muscle and often abdominal muscles.

The consequent alteration in the volume of the abdominal cavity produces pressure changes which are referred to the internal pressures of abdominal viscera. Thus any hollow viscus within the abdominal cavity will be influenced by the intra-abdominal pressure but will also exert independent pressure on its own contents. The fluctuations in bladder pressure which were associated with respiration in these experiments were not, therefore, unexpected.

The elevation in pressure which occurred immediately before eructation was of interest. Several workers have studied the physiology of eructation in ruminants in the last decade and Dougherty (1961) has reviewed existing knowledge in this field. Little attention has however been given to the intra-abdominal pressure changes associated with eructation. Although experimental evidence in the present work is meagre it indicates that eructation is probably assisted by a rise in intra-abdominal pressure resulting presumably from contraction of abdominal or diaphragmatic musculature.

The sharp rise in intracystic pressure which occurs with the detrusor response to bladder filling did not occur under epidural anaesthesia (p. 53). This response was, however, one of the first to return

as anaesthesia wore off and the nerve routes mediating the reflex became functional. Accurate measurement of the intracystic pressure was impossible because of the movement of the cow. The marked arching of the back altered the angle of the pelvis, the height of the external urethra and the position of the bladder. It may be that the characteristic action and posture of the cow at micturition can be inhibited by prevention of back arching, and it is possible that this postural change is necessary to assist the detrusor response of the bladder wall in overcoming the negative intracystic pressure and expelling the bladder contents. Further study of this phenomenon is contemplated.

In two of the three experiments intended to show the effect of epidural anaesthesia on the intracystic pressure, no effect was noted. This is in accordance with the findings of Nesbit & Lapides (1947, 1948) who found that chemical blockade of the autonomic ganglia in dogs did not decrease the tone of the bladder, or its ability to accommodate increasing volumes of fluid at the same intracystic pressure, though voiding contractions were abolished. There was no evidence to support the early view of Cuillé & Cholle (1931) that during epidural anaesthesia the bovine bladder becomes contracted. It is, therefore, unlikely that the use

of epidural anaesthesia in the cow should effect cystometric measurements except for abolishing the detrusor response. Without the use of anaesthesia, the stimulus of a urethral catheter causes excessive and often continuous attempts to micturate and cystometric measurements become impossible. The effects of epidural anaesthesia, therefore, appear to be solely beneficial in bladder or renal function studies.

The demonstration of a negative intra-abdominal pressure was not unexpected. Laparatomy in the standing cow is usually accompanied by an apparent equalisation of intra-abdominal and atmospheric pressures, though it is often not clear whether air is entering or leaving the abdominal cavity. As it is difficult to imagine any function of abdominal muscle tonus maintaining a negative intra-abdominal pressure, it is probable that the anatomical structure of the bovine abdominal wall is responsible for this phenomenon.

The negative pressure in the abdominal cavity must contribute to the negative pressure in the bladder and other abdominal viscera, but contraction of visceral walls will, of course allow pressure changes which are independent of intra-abdominal pressure.

SUMMARY

1. The measurement of intracystic pressure and the variation under different conditions were described.

2. The pressure of the empty bladder was in all observations found to be less than atmospheric pressure. The values found usually ranged between -5 and -10cms of water.

3. The effect of intra-abdominal pressure changes and the pressure responses of the bladder to filling were recorded.

4. The findings were discussed, and it was concluded that under the circumstances described, bovine bladder pressure was usually sub-atmospheric and that this phenomenon was related to the existence of a negative intra-abdominal pressure.

(d) Urinary 'delay time'

The time which elapses between the injection of a filterable substance into the blood stream, and its first detectable appearance in the urine is called the 'appearance time'. In this space of time the substance is carried by the circulation to the glomeruli of the kidney, filtered into Bowmans capsule, and transported by renal tubules and ureters to the bladder. Similarly, if the concentration of a substance in the plasma is increasing, or decreasing, time elapses before the result of this alteration in concentration is quantitatively reflected in bladder urine. This has been called 'the delay time'.

If the plasma concentration alters continuously, equilibrium between bladder urine, and plasma is never established. Michie & Michie (1951) found that in man clearance values and plasma concentrations are not in equilibrium until 20 minutes after starting the infusion leading to a constant plasma concentration. They attributed this delay primarily to 'the time required to wash out the dead space of the renal tubules and the renal pelvis, new urine being mixed with old until the latter is completely replaced'.

The delay time is longer than the first appearance

time which only marks the arrival of the most rapidly moving particles of the injected substance.

In view of the fact that initial inulin clearance measurements were made using a single injection technique dependent on a falling plasma inulin concentration, it was necessary to measure the urinary delay time in the cow.

The only record of delay time in cattle was that determined by Poulsen (1957) in which he found that 'appearance time' averaged 200 secs in 6 cows. By using a clearance technique 'delay time' was found to be twice the 'appearance time'. The delay time found was, therefore, 6 mins.

METHODS AND RESULTS

Three experiments were carried out on three cows using a solution of phenol red in saline as the marker dye. 50ccs of this solution was injected rapidly into the jugular vein, and a stop watch started in the middle of the injection. The time when the dye first appeared in the translucent collection tubing attached to the catheter was taken to be the first appearance time. The first appearance times and the mean urine flow rate during each measurement are shown on table 8. The mean first appearance time was 162 secs or about

Table 8

The length of time between injection of phenol red into the jugular vein and its first appearance in the bladder drainage tubing.

Mean urine flow rate (ml/min)	First appearance time (sec)
4.0	120
5.4	195
8.0	170
	Mean 162

2½ minutes. There was no relationship between rate of urine flow, and the length of the first appearance time.

DISCUSSION

The absence of a correlation between rate of flow and first appearance time has been noted in the dog (Morales, Crowder, Fishman, Maxwell & Gomez, 1950).

The mean first appearance time found (162 secs) was rather less than found by Poulsen (1957) but there was a wide range over the 3 experiments. In view of the fact two of the results were within 15 secs of the delay time found by Poulsen, it was decided to use Poulsens figures for first appearance time, and delay time, i.e. 3 mins, and 6 minutes respectively.

SUMMARY

1. Three measurements of the first appearance time of Phenol Red in three cows were made.

2. The mean first appearance time was 162 secs.

3. As two of the three results were within 15 secs of the figure of 3 mins used by Poulsen (1957) it was decided to base estimates of urinary first appearance time and delay time on his findings i.e. 3 mins and 6 mins respectively.

(c) The technique and accuracy of urine collection

It is generally accepted that one of the main sources of inaccuracy in studies of renal function in the unanaesthetised subject is the difficulty of obtaining complete bladder drainage (Smith 1951). The importance of an uncollected pool of urine in the bladder increases as the duration of the collection period is shortened. A small volume of uncollected urine is of little importance when daily outputs are under consideration, but may cause a large error when the collection period is shortened to 15 minutes.

Most investigators of renal function in unanaesthetised cows mention urine collection methods only briefly and say nothing of the difficulties of accurate collection of urine over relatively short periods of time. Sellers & Roepke (1951a) collected urine from cows over two hour periods, but did not give details of the catheter used. Cunningham et al (1955) described a self retaining rubber urethral catheter with an inflatable bulb which was employed for the continuous collection of urine over long periods (up to three weeks), and they reported that some cows show slight discomfort when first catheterised. Sellers et al

(1958) mentioned a soft rubber catheter secured to wobbling straps for urine collection during clearance periods of 15 to 30 minutes duration. Poulsen (1957, 1959) and Knudsen (1960) used a balloon catheter (Rusch or Acmi No. 22 - 26) with a balloon volume of 75 ml. The outflow tube of the catheter was closed by a clamping screw which was opened at the end of each clearance period. By pulling the catheter to the rear at the end of the period, contractions of the bladder and abdominal muscles were elicited, and the urine emptied into the collecting vessel. De Groot & Aafjes (1960) reported inconsistent results when they used urethral catheters in adult cattle, with the additional disadvantage of occasional damage to the urinary tract, and they adopted a special apparatus described by Van Es & Vogt (1959), in which catheterisation was avoided.

Modifications of the Foley catheter (Warne, 26 F.G.; 100 ml bulb capacity) were used by the author in attempts to establish an accurate method of urine collection from conscious cows during 15 to 30 minute periods. Though satisfactory drainage may be achieved with such a catheter - particularly at high rates of flow - continuous urine collection often became irregularly intermittent, giving markedly

inconsistent measures of urine formation. This was attributed to occlusion of the perforations of the catheter by folds of the mucous membrane of the bladder when contracted and empty.

Two other difficulties were experienced. First, the negative (sub-atmospheric) pressure which is a normal feature of the bovine bladder resulted in an inrush of air on catheterization which made the establishment and maintenance of drainage by siphonage difficult. Second, introduction of a catheter into the urethra of an unanaesthetised cow invariably caused quite marked evidence of discomfort, and, once introduced, the inflated retaining cuff resting against the internal urethral orifice caused frequent, and sometimes continuous attempts to micturate. Urine was expelled past the catheter, and the catheter itself often expelled. The cow defaecated frequently soiling the catheter and collection tubing, and showed other manifestations of discomfort. In addition to the inconvenience of this behaviour, such features of an experiment must be considered undesirable, because of the effects of stress on urine flow of the cow (Author, 1961). A technique to eliminate the above difficulties is described and evidence of its accuracy is presented.

The technique of bladder drainage by the 'Ramshorn' catheter

A moulded rubber catheter with a coiled multi-perforate tip was used ('Ramshorn'; Vicson 14 E.C.). For introduction, this catheter was straightened with a stainless steel stilette, and the coiled tip retained the catheter *in situ* when the stilette was withdrawn. As the perforations were in the inner aspect of the coiled tip, they could not be occluded by the mucous membrane of the bladder. Introduction of the catheter was facilitated by use of a sterile, water-soluble lubricating jelly (Johnson and Johnson 'KY' Lubricating Jelly).

A tightly fitting rubber cuff around the stilette prevented air being sucked into the bladder when the catheter was introduced. The stilette was partly withdrawn once the tip of the catheter was in the bladder, and the catheter clamped before the stilette and cuff were completely removed. The catheter, stilette and cuff are shown in Fig. 7.

With the catheter in place, polythene drainage tubing was attached, and the bladder emptied by establishing siphonage. Urine was then collected continuously.

Discomfort during catheterization and during urine collection, and attempts to micturate and

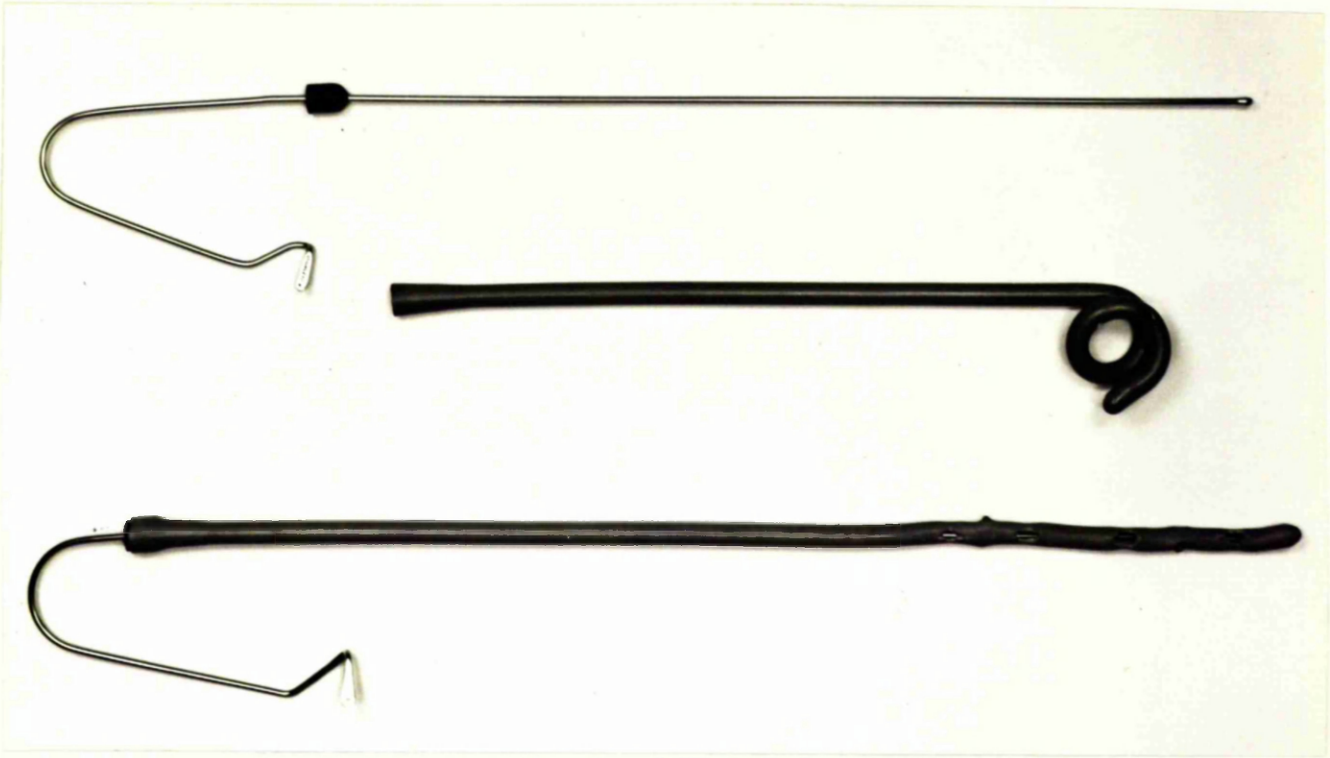


Fig. 7. The "Ramshorn" urethral catheter. The stilette with rubber cuff and the catheter are shown separately at the top, and catheter with stilette and rubber cuff in place are shown at the foot of the illustration.

defaecate, were eliminated by prior induction of posterior epidural anaesthesia effected by the injection of 4 ml. of 5 per cent procaine solution (May and Baker 'Planocaine') through the sacro-coccygeal space. When an experiment lasted longer than two hours, epidural anaesthesia was maintained by additions of 3 or 4 ml. of the anaesthetic when signs of returning sensation occurred.

Experiments to determine the accuracy of urine collection

While most workers are aware of the necessity of accurate urine collection in renal clearance measurements, there have been few attempts to assess the accuracy of a particular method of urine collection in cattle, or in other species. The experiments described below were used to determine the reliability of various catheters in bladder drainage.

The measurements are in two categories:

(a) the effect on the rate of urine collection of superimposing an inflow of known rate into the bladder,

(b) measurement of inulin clearance over a wide range of urine flow.

(a) The effect on the rate of urine collection of superimposing an inflow of known rate into the bladder

If urine collection by an indwelling urethral

catheter is efficient, then any alteration of the rate of inflow into the bladder from the ureters must be mirrored exactly by a corresponding alteration in the rate of urine flow from the catheter. It is not possible to induce a known alteration of inflow into the bladder by causing a diuresis. While an increase in the rate of collection may result from the diuretic stimulus, inefficient bladder drainage may not reflect this increase with quantitative accuracy. If, however, fluid is infused into the bladder from an external source during urine collection, this superimposed inflow may be measured and controlled, and the increase in the over-all outflow be compared with the total input. Clearly, output must equal input over a period of several hours, but incomplete drainage may cause significant errors in a 15 min clearance period.

In three experiments, the rate of urine collection from the bladder was augmented by an infusion of distilled water at a known rate. This technique proved useful, but had the disadvantage that it was dependent on the constancy of the ureteral input during the period of infusion. Should the actual rate of urine flow alter over this period, then the respective contributions of the distilled water and urine to the total output could not be measured.

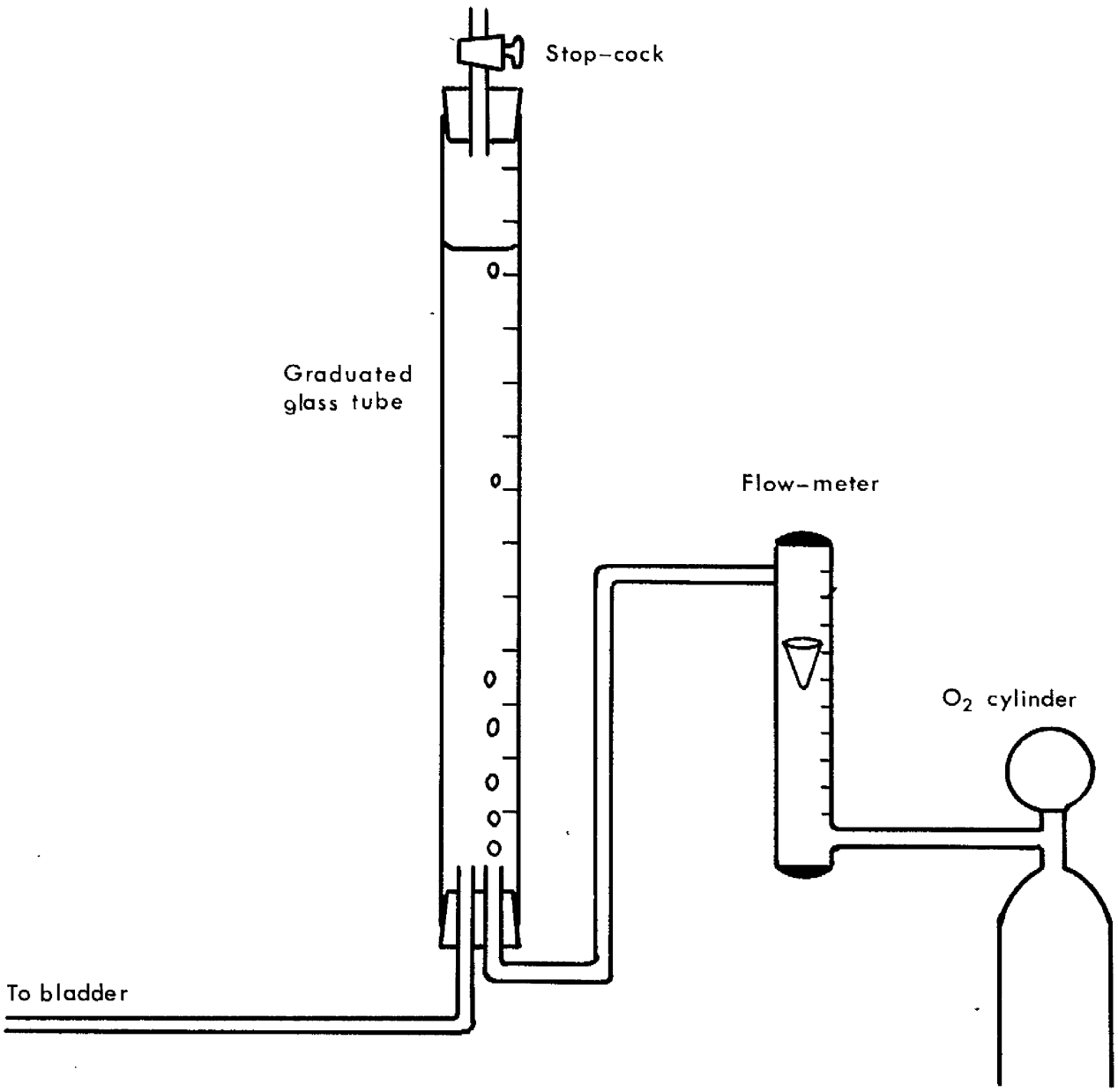


Fig. 8. Diagrammatic illustration of apparatus used for infusing water at a constant controlled rate into the bladder.

Measurement of the concentration of a urinary constituent such as creatinine would be of value provided its output remained constant, but it was decided to infuse a dye at known concentration and rate on the grounds of precise control of administration and ease of estimation. The dye used was Evans Blue (T. 1824).

The apparatus used to test the reliability of the Warne Balloon Catheter by infusion of distilled water is shown in Fig. 8. This apparatus with minor modifications was used for water infusion experiments I to III.

In Experiment IV to determine the reliability of the 'Ramshorn' catheter a micropump was used for controlled infusion.

A solution of Evans Blue (T. 1824) containing 3.4 mg/100ml. was infused into the bladder by the micro-pump calibrated to deliver 17.8 ml./min. The volume of dye actually delivered by the pump was checked at 5 min intervals. The dye was infused for 60 min.

The concentration of dye in the samples was estimated by an E.E.L. colorimeter using a 607 filter. A urine blank, dye standard, and urine samples were made up by the following procedure:-

Urine blank: 2 ml. urine blank + 6 ml. normal saline

Standard: 2 ml. urine blank + 5 ml. normal saline + 1ml. dye standard.

Urine Sample: 2 ml. urine sample + 6 ml. normal saline.

The concentration of Evans Blue in the urine samples was calculated by simple proportion from the dye standard, with corrections for dilution and the urine blank. The volume of urine collected in each period of infusion was calculated from the dilution of the dye.

The measurements made were of volume collected (ml.) and dye concentration (mg/100ml.), and hence the amount of dye recovered was calculated (mg).

On the assumption that there was no significant fluid movement across the bladder wall, nor any significant dye uptake, the actual volume (ml.) of the dye solution recovered was calculated from the amount (mg) recovered. As the experiment was concerned with volume recoveries, the results are expressed as volumes (ml.).

Urine volumes were measured for two 15 min periods before infusion and a sample for blank analysis taken. During the 60 min infusion period volumes were measured and samples taken every 5 mins for the first 15 min, and every 15 min thereafter. After infusion, 5 min volumes and samples were taken for 40 min.

All experiments were carried out using the author's experimental cows, and the described techniques of handling and catheterisation.

RESULTS

Experiment I

A Foley catheter was used for urine collection. (Fig. 9). The volume of water infused into the bladder was measured at 5 min intervals, but it was not regulated. Urine flow was measured at 5 min intervals for 25 mins before infusion was begun. 500 ml. of distilled water at 38°C was then infused over 25 min and the outflow of the bladder was measured at 5 min intervals.

The results which were obtained are shown in Fig. 10 The features of the experiment are that, after a fairly constant preliminary urine collection period, the volume collected over 5 min intervals rose from 140 ml. to 182 ml. - a rise of 42 ml. - during the first period of infusion. It then rose to a maximum of 248 ml. at 15 min and declined to 164 ml. at 25 min. The volume during the first post infusion period was 75 ml. Thus the true urine flow fell from 140 to 75 ml./5 min during the period of infusion. This marked fall in the true urine flow over the infusion

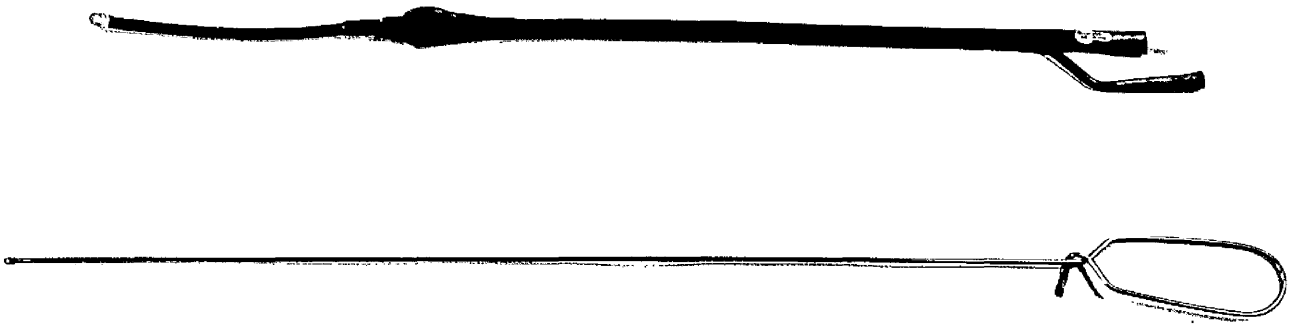


Fig. 9. Foley catheter (Warne, 26 F.G. 100 ml bulb capacity) with multiperforate extension.

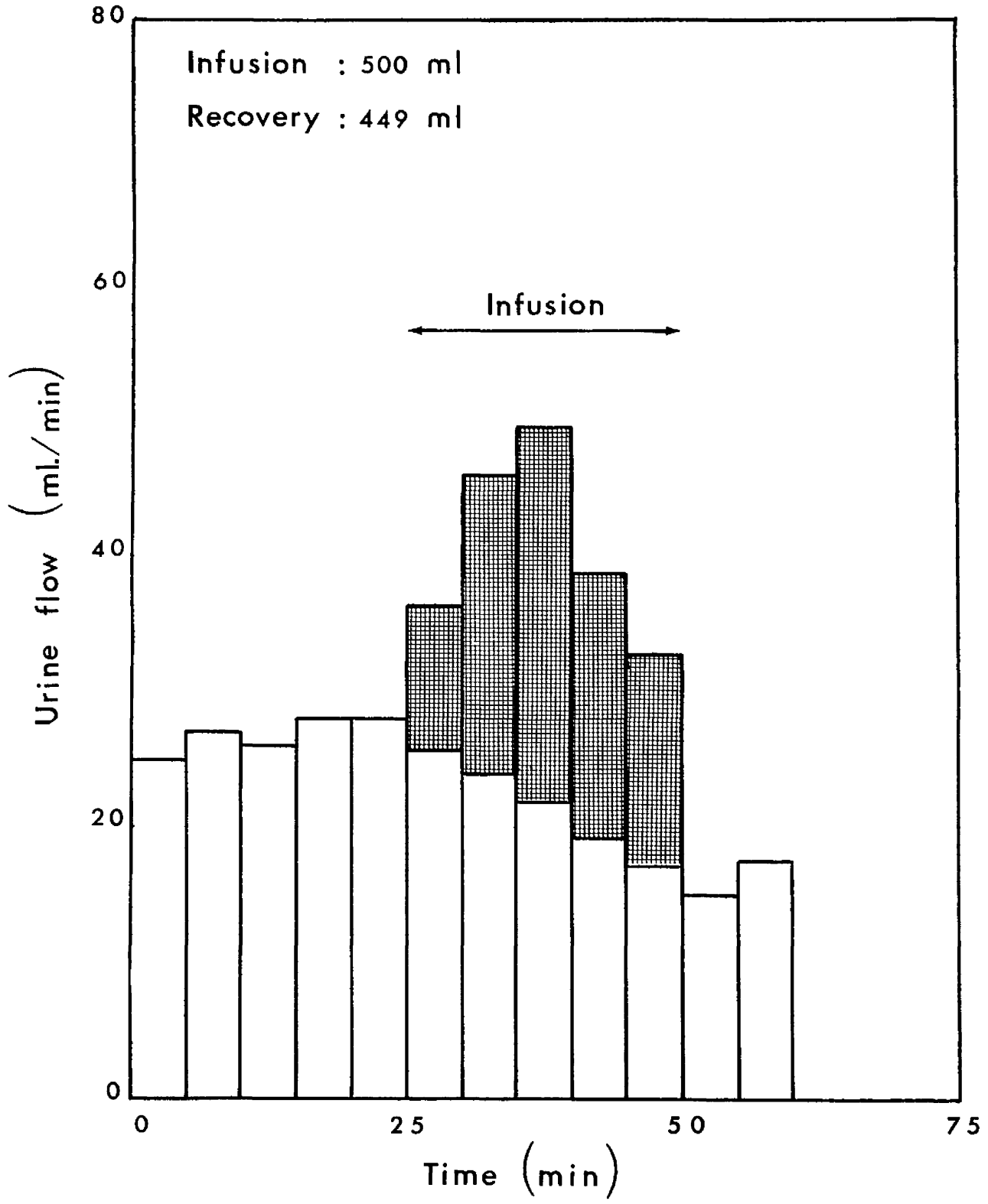


Fig. 10. Urine flow measurements during bladder infusion with distilled water. The hatched area represents the increase in flow rate due to the infusion, assuming that actual urine flow showed a constant decrease between the last pre- and the first post-infusion periods.

period prevented accurate interpretation of the results.

In an attempt to obtain some information from the experiment, it was assumed that the true urine flow decreased at a uniform rate from 140 to 75 ml./5 min over the infusion period. This is shown on Fig. 10 below the hatched area. The hatched sections immediately above therefore, represent the increase due to the infusion. The total volume of the latter was found to be 449 ml. Thus 51 ml. (10%) of the 500 ml. infusion was uncollected. When the individual 5 min volumes represented by the unhatched areas are compared with the volumes infused (Table 9), the discrepancies are very much greater. These discrepancies are probably exaggerated by the fact that the assumption of a uniform decrease in urine flow during infusion was probably unjustified. In this particular experiment, the infusion of a dye instead of distilled water would have removed many of the uncertainties. There was, however, sufficient evidence to indicate that there was a considerable volume (90 - 100 ml.) of urine uncollected in the bladder, and that variations in the volumes collected probably bore little relationship to actual variations in urine flow.

Experiment II

Urine flow remained constant throughout the

Table 9

Comparison of the volumes infused into the bladder and the volumes collected in excess of the estimated urine flow during 5 min collection periods.

Experiment I

Period	Vol. infused (ml/5 min)	Increase in rate of collection (ml/5 min)
1	75	49
2	75	108
3	100	138
4	100	97
5	150	58
Total	500	449

preliminary collection period, and apparently maintained its constancy during and after water infusion. Consequently, the effect of the infusion was much more clear-cut (Fig. 11). In this experiment, the relationship between the volume infused, and the increase in the mean urine flow was slightly better than in Experiment I; 500 ml. infused, 465 ml. recovered (Table 10). As in the previous experiment there were discrepancies - though less marked - between the actual volume infused and the resulting increase in urine flow during 5 min periods. Assuming the maintenance of constant urine flow during the period of infusion, there appeared to be a delay of about 15 min before the increase in output overtook the infusion rate.

Experiment III

There was little apparent change in flow rate between the last pre-infusion period and the first post-infusion period, though subsequent periods showed a lower rate. (Fig. 12). Assuming a constant urine flow of 19 ml./min between the last pre-infusion period and the first post-infusion period, the increase in the volume collected (hatched area) was 510 ml. during the 30 min when 500 ml. was infused. Urine flow during this time must therefore have exceeded the

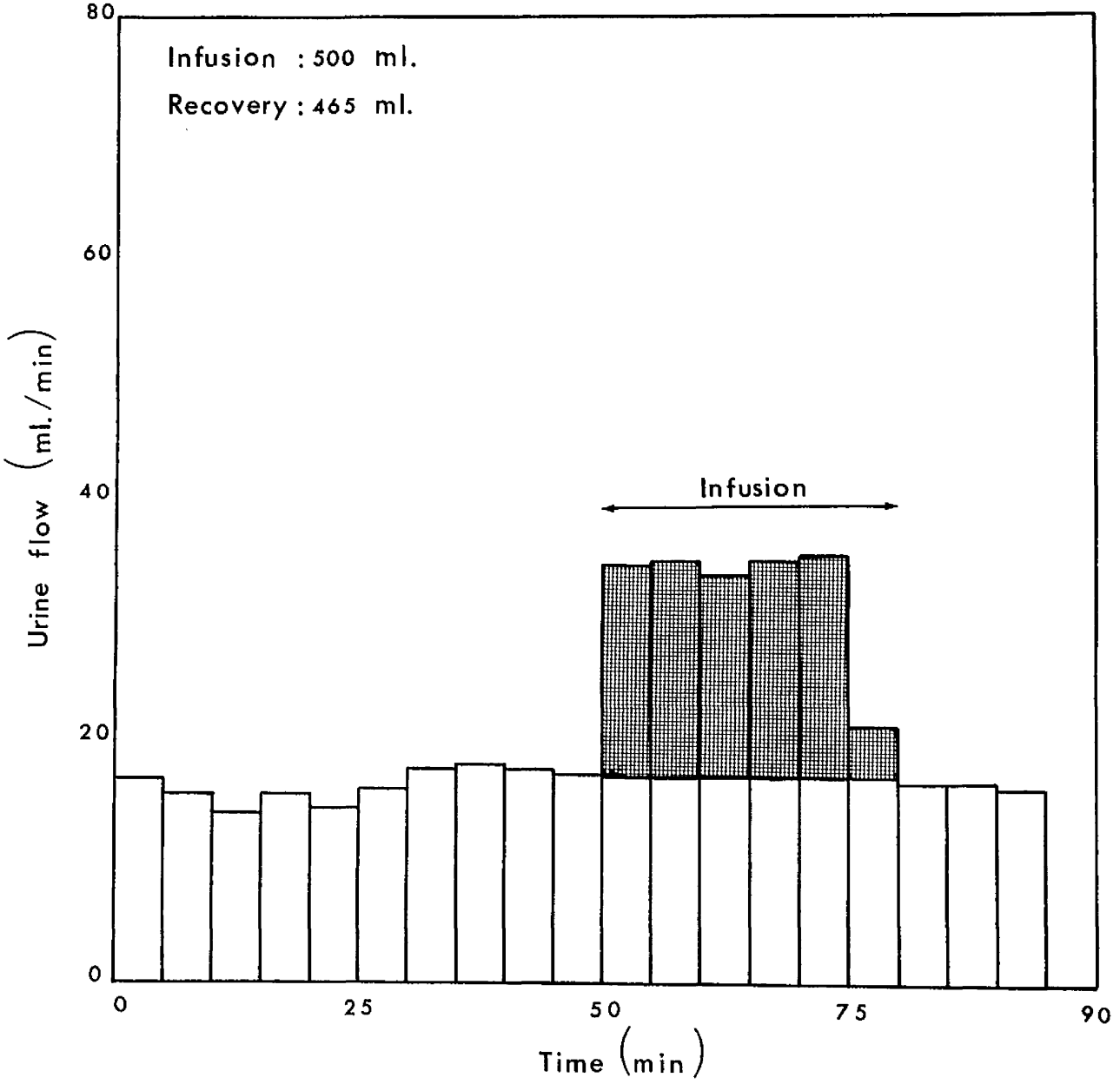


Fig. 11. Urine flow measurements during bladder infusion with distilled water. The hatched area represents the increase in urine flow rate due to the infusion, assuming that the actual urine flow maintained a constant rate between the last pre- and the first post-infusion periods.

Table 10

Comparison of the volumes infused into the bladder and the volumes collected in excess of the estimated urine flow during 5 min collection periods.

Experiment II

Period	Vol. infused (ml/5 min)	Increase in rate of collection (ml/5 min)
1	100	88
2	100	90
3	90	84
4	85	90
5	85	91
6	40	22
Total	500	465

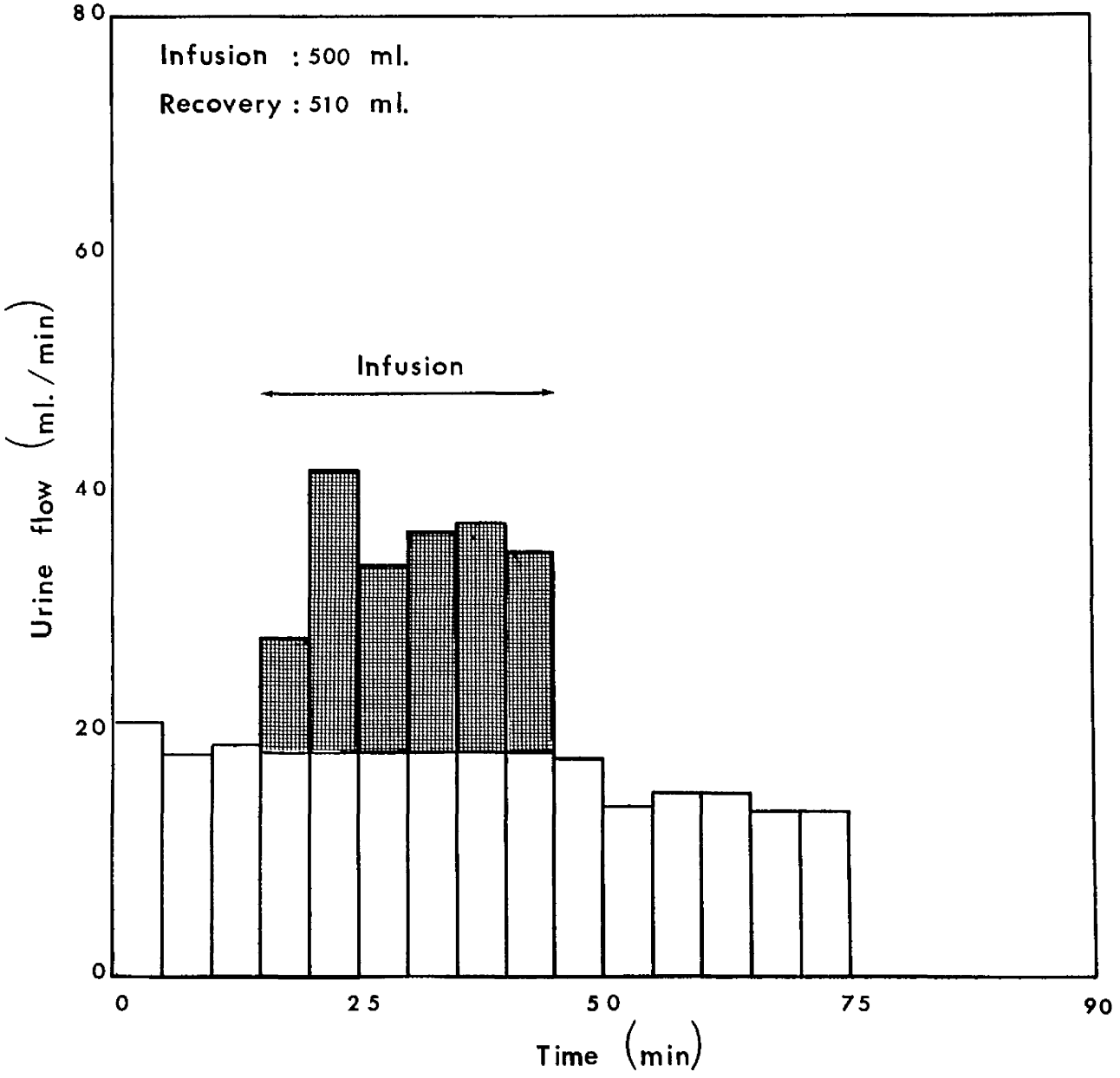


Fig. 12. Urine flow measurements during bladder infusion with distilled water. The hatched area represents the increase in urine flow rate due to the infusion, assuming that the actual urine flow maintained a constant rate between the last pre- and the first post-infusion periods.

assumed rate of 19 ml./min. The discrepancies between the volumes infused and the estimated increase in each period are shown in Table 11. In this experiment the increase in output overtook the infusion rate after 10 min and there is good agreement between the sum of the increases in the first two collection periods (164 ml.) and the amount infused (170 ml.), and in the last two collection periods (177 ml.) and the amount infused (175 ml.).

Experiment IV

The rate of urine flow was constant for 30 mins before dye infusion. Flow rate was markedly increased from the first 5 min period of infusion. The dye appeared in the collection tubing about 30 secs after the start of infusion, and in the collection vessel after a further 30 secs. Collection rate further increased to the middle of the infusion period and then declined during the latter half. In the first post infusion periods, flow rate was much less than the pre-infusion rate. In Fig. 13 the volume of dye recovered is shown as the hatched area, the area below the hatching being the calculated urine flow during each period.

A total volume of 1,061 ml. of dye was infused in 60 min giving a mean rate of infusion of 17.7 ml./min.

Table 11

Comparison of the volumes infused into the bladder and the volumes collected in excess of the estimated urine flow during 5 min collection periods.

Experiment III

Period	Vol. infused (ml/5 min)	Increase in rate of Collection (ml/5 min)
1	85	47
2	85	117
3	90	78
4	65	91
5	85	95
6	90	82
Total	500	510

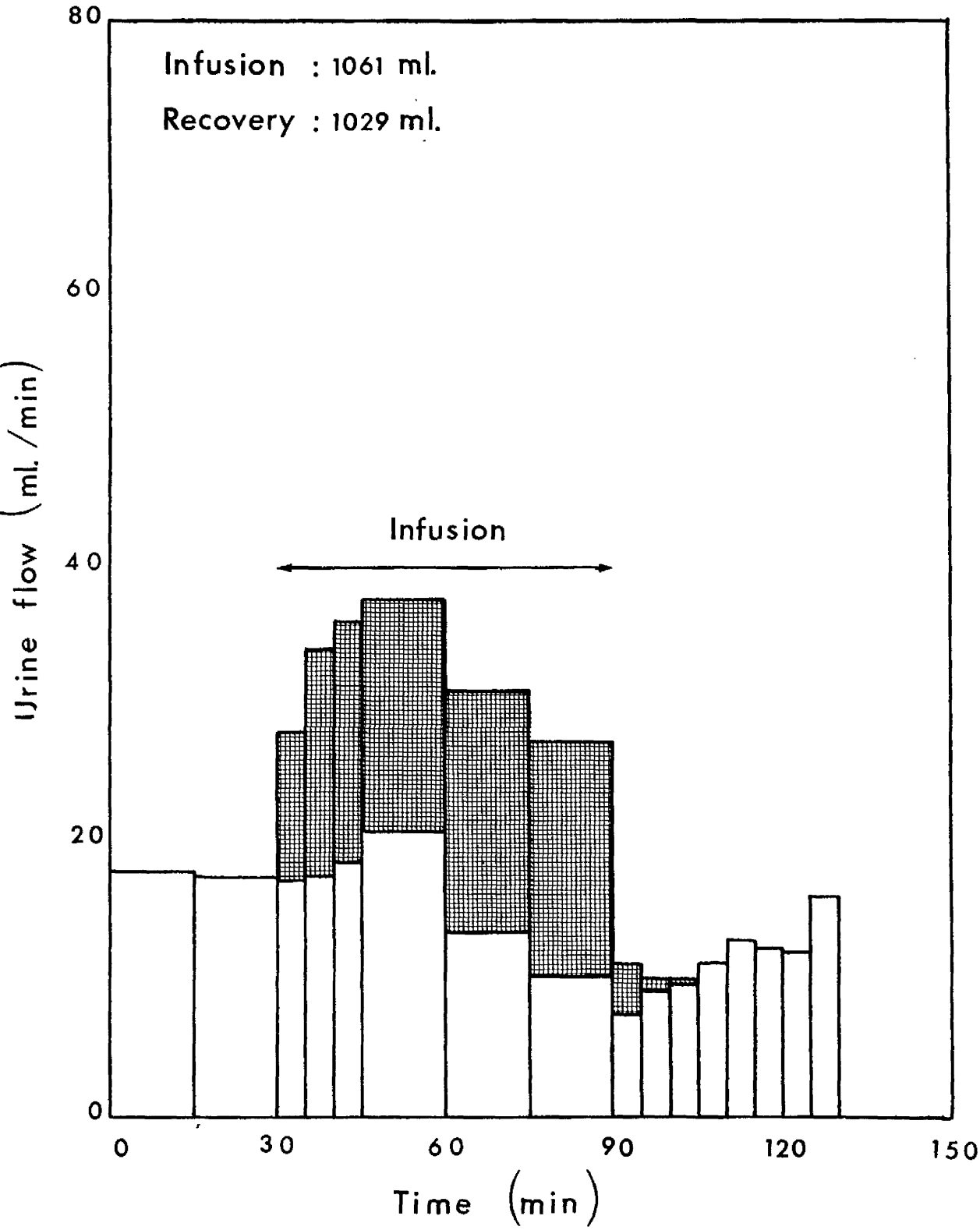


Fig. 13. Urine flow measurements during bladder infusion with a solution of Evans Blue dye (T.1824) containing 3.4 mg/100ml. The hatched areas represent the volume of dye recovered during each period.

At 65 min 1,021 ml. (96%) of the dye had been recovered. The total recovery was 1,029 ml. at 100 min (97%) (Table 12). Thus 32 ml. was not recovered. As no dye was present in the last samples, it is probable that most of this dye was fixed to the bladder mucous membrane. If this volume is discounted, then the volume of free dye in the bladder 5 min after the end of infusion was 40 - 32 = 8 ml. and the percentage recovery $\frac{1053}{1061} \times 100 = 99\%$.

A comparison of the rates of inflow and outflow in the individual periods is shown in Table 13. Urine flow during dye infusion was calculated from the dilution of the dye at the end of each period. After 5 min (period 0 - 5) the rate of outflow was substantially lower than the calculated rate of inflow, but in all subsequent periods there was good agreement between the two rates.

After the end of the infusion the bladder was quickly cleared of the remaining dye. In Fig. 13 it can be seen that most (18 ml.) of the residual dye was collected during the first 5 min after infusion and only traces were present after 15 min. The difference between the volume of dye in the bladder at the end of infusion (49 ml.), and the volume of dye which was

Table 12

Volumes of Evans Blue dye recovered from the bladder during simultaneous infusion and collection.

Experiment IV

Period of infusion (min)	Vol. infused (ml)	Vol. collected[x] (ml)	Uncollected Dye (ml)
5	87	62	25
10	176	146	30
15	262	229	33
30	528	484	44
45	793	749	44
60	1061	1012	49
65	1061	1021	40
70	1061	1025	36
100	1061	1029	32

Dye recovery at 100 min = $\frac{1029}{1061} \times 100 = 97\%$

[x] Total volume collected includes the estimated volume in the catheter and collection tubing at the end of each period.

Vol. of catheter and collection tubing = 17 ml.

Vol. of dye in catheter $= \left(\frac{\text{concn. in sample}}{\text{concn. of infusion}} \times 17\right)$ ml.

Table 13

The rate of inflow into the bladder (urine and dye) and the rate of collection of mixed dye and urine.

Period (min)	Rate of inflow		Total rate of inflow (ml/min)	Rate of collection (ml/min)
	Infusion (ml/min)	Urine flow[x] (ml/min)		
0 - 15	-	17.9	17.9	17.9
15 - 30	-	17.5	17.5	17.5
30 - 35	17.3	17.0	34.3	28.0
35 - 40	17.8	17.4	35.2	34.0
40 - 45	17.3	18.4	35.7	36.0
45 - 60	17.7	20.6	38.3	37.7
60 - 75	17.7	13.4	31.1	31.0
75 - 90	17.9	9.9	27.8	27.3
90 - 95	-	11.0	11.0	11.0
95 - 100	-	10.0	10.0	10.0

[x] During dye infusion and collection, urine flow rate was calculated from the dilution of the dye at the end of each period.

apparently irrecoverable (32 ml.) represented the dead space or the uncollected fluid in the bladder (17 ml.)

DISCUSSION

In experiments I to III, there was an immediate increase in the rate of outflow from the bladder in response to the increased inflow. The increased outflow did not, however, quantitatively match the increased inflow for the first 10 to 15 min. This suggested that the sudden increase in input resulted in a temporary increase in the uncollected pool of urine in the bladder. Thus a rapid diuretic response, though qualitatively apparent almost immediately, may be quantitatively inaccurate for 10 to 15 min after its onset. The relationship between the total volumes of water infused and the total increase in output in experiments I and III is not of great value due to the marked alterations in true urine flow during the period of infusion. In experiment II, true urine flow was the same at the beginning and end of infusion, thus the response to the infusion was more clear cut. The discrepancy of about 7% between the volume infused and increase in collection is acceptable in clearance techniques. These experiments were made using a

Warne balloon catheter, and the conclusion reached was that it was unreliable for short term clearance experiments as a result of its failure to show quantitative changes in urine flow.

In Experiment XV the 'Ramshorn' catheter and the dye recovery method produced more reliable results than in the previous experiments. The infusion of the dye allowed accurate calculation of the true urine flow during the infusion, the delay between the appearance of the first particles of the dye in the bladder and its appearance in the catheter, the percentage recovery of the dye, and the length of time after input ceased that residual amounts of the dye appeared in the collection apparatus.

The results indicated that when a new substance appeared in the bladder there was a delay of about 60 secs before it was recovered by the collection apparatus. This time is of course dependent on the volume of the collection apparatus, and the rate of urine flow. In the present work, where the volume of catheter and tubing was 17 ml, the calculated time for the dye to flow from the bladder to the collection vessel at a flow rate of 28 ml./min was about 36 secs, thus there was a delay in the bladder of about 24 secs.

There was also a delay in the quantitative response

of the rate of collection to an increased rate of input (Table 13). During the first 5 min of infusion, the input rate was increased by infusion from 17 ml./min to 34.3 ml./min. Despite this, the actual rate of collection only rose to 28 ml./min. The second and subsequent periods, however, showed a close relationship between the rate of input and the rate of outflow of the bladder.

The recovery 5 min after the end of infusion of 96% of the dye infused indicated that the residual volume of uncollected fluid in the bladder was about 40 ml. This was rather a large volume for accurate clearance studies, but when it became evident that about 32 ml. of this volume was not as free dye in the bladder, then the probable volume of uncollected dye was reduced to 8 ml. (99% recovery). In a viscus capable of containing several litres this indicated efficient drainage.

The measurable concentrations of the dye ceased to appear in the urine 15 min after the end of infusion. Thus, using the collection system described there was a delay of about 15 min between the time when the dye stopped entering the bladder and its disappearance from the collection vessel. Like the first appearance of the dye in the bladder this time is inversely related

to the rate of urine flow.

Most workers on renal clearance measurements induce a water diuresis in animals and man in order to reduce the errors arising from inaccuracies in the measurement of a low rate of urine flow. The results of the present study suggest that the same effect could be achieved by infusion of distilled water at a known rate into the bladder during urine collection. The output of all the urinary constituents would remain the same, and the actual urine flow could be calculated at any time by subtraction of the rate of infusion from the rate of collection. The main recommendation of such a technique is that the advantage of a diuresis to clearance studies is achieved without modification of renal function.

Measurement of inulin clearance over a wide range of urine flow.

Glomerular filtration rate remains fairly constant despite wide variations in urine flow (Smith, 1951). If, in the measurement of glomerular filtration rate, urine collection technique is inefficient, then constant values of glomerular filtration rate are not achieved. Failure to collect all urine from the bladder during one period results in an apparent fall in filtration rate, and if the uncollected urine is

released during a subsequent period, an erroneously high G.F.R. is then obtained. During diuresis, the output of a substance such as inulin should remain unaltered, and, in the presence of constant plasma levels the urinary concentration of inulin is inversely proportional to the urine flow. If the bladder is not drained of urine during a diuretic response, the reduction in inulin concentration is not matched by an increase in urine volume, thus the glomerular filtration rate which is measured is again erroneously low. It has been seen in the preceding section that there does appear to be a delay in the response of the rate of collection to alterations in inflow into the bladder. If this delay were important in clearance measurements, then the values of glomerular filtration rate during a sudden diuresis would not remain constant.

The results of three experiments in which a diuresis was induced by the intravenous infusion of hypertonic (molar) KCl at 7 to 9 ml./min, are shown on Table 14. In these experiments, when diuroses of up to 600% were induced, inulin clearance measurements remained constant within the accepted limits of 5 - 10%. (Fig.[18]).

The constancy of the values for glomerular filtration rate obtained in these experiments indicated

Table 14

Inulin clearances (G.F.R.) before and during diuresis.

Case 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	8.3	953
	50.0	1003
	37.7	924
	28.2	970
	22.9	995

that the urine collection method used operated efficiently over a range of 8 to 68 ml./min.

SUMMARY

(a) Experiments I to III were carried out to assess the accuracy of a Foley catheter (Warne, 26 FG, 100 ml. bulb capacity) in continuous urine collection. The results indicated that this catheter was unreliable in short clearance measurements in cattle, as a considerable volume (90 - 100 ml.) of uncollected urine was allowed to accumulate in the bladder.

Experiment IV utilised a dye infusion technique to assess the accuracy of a 'Ramshorn' 14 FG catheter. The results indicated this catheter drained the bladder efficiently, and that there was only a short delay in an accurate response to an artificially elevated inflow into the bladder.

(b) In a large number of inulin clearance measurements during which marked variations in the rate of urine flow were induced, there were few variations in inulin clearance values which could be attributed to inefficient urine collection.

The evidence presented for the accuracy of a urine collection technique is simply a guide to the performance which may be expected from a particular method of

urine collection. It does not allow the establishment of a value for percentage accuracy. In renal clearance studies, the accuracy of urine measurement depends on factors peculiar to the individual experiment - true variability of urine flow, very low rates of flow, poor anaesthesia, response of the animal to experimental interference, and other factors. It is not, therefore, possible to apply limits of error to one experiment as a result of previous measurements. The results of experiments such as have been described, do, however, allow some quantitative assessment of the merits of different methods of collection, and when a particular technique is frequently accurate, other results which are dependent on this method may be accepted with some confidence.

Section 3

Measurement of inulin clearance by a single injection method

Measurement of inulin clearance by a single injection method

Measurement of the renal clearance of inulin is accepted as the experimental method of assessing glomerular filtration rate in man (Smith, 1951, Kennedy & Kleh, 1953), despite criticism of its validity by Ferguson, Robson, Olbrich & Stewart (1949) and Wolf (1950). Although inulin clearance has been extensively examined in man, dog and laboratory animals, until recently there are few records of its use in large domestic animals. Shannon (1937), and Sperber & Sperber (1955) investigated inulin clearance in the sheep, and it has been generally accepted and used as a measure of glomerular filtration rate in this species.

Several workers have used inulin clearance techniques in the cow. Poulsen (1957) measured the renal clearance of inulin, creatinine, thiosulphate, urea and diodrast. His findings satisfied most of the criteria suggested by Smith (1951) for the validity of inulin clearance as a measure of glomerular filtration rate. Sellers *et al* (1958) used inulin clearance as a measure of glomerular filtration rate in renal function studies in calves and heifers, and Anderson & Mixner (1960) described a method for measuring inulin

clearance by studying the rate of disappearance of inulin from the plasma after a single injection. Vogel (1959) and Ketz (1960) have studied renal function in several species of domestic animals including the cow using both single injection and constant infusion techniques.

In the present study, some initial difficulty was experienced in accurate measurement of the rate of urine flow. It was therefore, decided to use a method which did not require accurate urine flow measurements. Several such methods have been published (Barnett, 1940; Earle & Berliner, 1946; Robson, Ferguson, O'Brien & Stewart, 1949). It was decided to use the method of Robson et al, which had the additional advantage that continuous infusion of inulin was not necessary. This method was, therefore, adapted for use in the cow.

METHOD

Robson and his co-workers invoked the following theoretical considerations in establishing their method. When inulin is introduced into the blood stream, it becomes distributed in a volume approximately equal to the extracellular space. It is excreted from the body solely by the kidneys at a rate proportional to the plasma concentration. Several workers have

claimed that when inulin was injected into the blood stream, it became evenly distributed throughout a 'volume of distribution', and that equilibrium between vascular and extra-vascular fluid was then maintained during continued excretion of inulin by the kidneys. The 'volume of distribution' is defined as that volume which would contain all the solute present in the body if it were evenly distributed at the concentration found in the plasma. This value may be expressed thus:

$$V = \frac{IB}{p}$$

where V = the volume of distribution of inulin
IB = the total amount of inulin in the body
p = the concentration of inulin in the plasma water.

If the concentration of the solute is in fact uniform throughout the fluids which contain it, the function V has real significance. If it is not uniform, V will bear no relationship to the volume of any actual fluid compartment. In either case, the volume of distribution as defined will be constant so long as equilibrium is maintained between vascular and extra-vascular fluids, but it cannot be constant otherwise. If equilibrium does not exist, then the rate at which the value of the expression $V = \frac{IB}{p}$ changes provides a value for the change in the amount of inulin in the

extra-vascular compartment relative to the plasma water concentration with respect to time.

It was shown that, after a single injection of inulin in 9 human volunteers, the function V showed an increase with time throughout the period of experiment up to 150 min, and it was concluded that, following intravenous injection of inulin, equilibration of distribution between plasma, and extra-vascular fluids did not occur. Having justified the assumption that the function V was linearly related to time the authors derived an equation for the rate of disappearance of inulin (renal clearance) making allowance for the linear increase in V with time,

$$C = \frac{b(\log p_1 - \log p_2)}{\log V_2 - \log V_1} \cdot b$$

where C = clearance in terms of plasma water
b = the constant of the straight line $V = a+bt$ which joins the values V_1 and V_2
p_1 and p_2 = the concentration in plasma water at times t_1 and t_2
V_1 and V_2 = the function $\frac{U}{P}$ at times t_1 and t_2

The constant b of the line joining the two values V_1 and V_2 was obtained as follows:-

The straight line joining the two points V_1 and V_2 is identified by the equation $V = a + bt$. Take the two points on the line t_1 V_1 and t_2 V_2 at times t_1 and t_2 respectively, then $V_1 = a + bt_1$

and $V_2 = a + bt_2$

$$\text{thus } b = \frac{V_2 - V_1}{t_2 - t_1}$$

In the use of the formula, it was convenient to employ values for V_1 and V_2 expressed in terms of hundreds of ml. of plasma water per min. This was converted to the usual units of ml. of plasma per min by multiplication of the 'C' value by the factor

$$\frac{10,000}{100 - g \text{ of plasma protein/100 ml.}}$$

Experimental Procedure

Six non-lactating, non-pregnant Ayrshire cows were used as experimental subjects. These animals were for part of the time on routine hospital feeding (p. 16.) and part of the time at grass. They were not forcibly hydrated before or during the experimental period, but had unrestricted access to water prior to experiment, and were occasionally offered water during the experimental period. All were clinically healthy during the period of experimentation. Restraint was applied as described (p. 16.) and the perineal area anaesthetised with epidural anaesthetic (p. 49.). Various techniques of urine collection were used. In early experiments, catheterisation was carried out by Nielsen's catheter just before the end of each collection period. This procedure did not, however, prove reliable for complete bladder drainage. Latterly a

retention catheter (Warne, 26 FG: 100 ml, bulb capacity) was placed in the bladder and urine collected at the appropriate time.

The jugular veins were catheterised with nylon catheters to facilitate injection of solutions and withdrawal of blood. A small area of skin over the jugular vein on each side about 20cm. below the angle of the jaw was clipped and swabbed with Tincture of Cetrimide B.P.* Each site was anaesthetised by subcutaneous infiltration with 6 ml. of 2% w/v procaine hydrochloride with adrenaline+. With the animals head restrained, a 18G x 1½in. Record fitting needle was introduced into the vein and 30cm of nylon rod (1.5mm diameter) fed through the needle into the vein. About 20cm. of nylon tubing (internal diameter 1.5mm) was then slipped over the rod into the vein, and the rod withdrawn. The tubing was then fitted with a rubber tubing adapter for a Record syringe, filled with heparinised saline, clamped off, and fixed in place (Fig. 10).

Inulin was prepared for injection as a 10% w/v solution in sterile saline and injected at 39°C. The volume given was based on an approximate dose rate of

* Tincture of 'Cetavlon' - I.C.I.

+ 'Planocaine' - May and Baker.

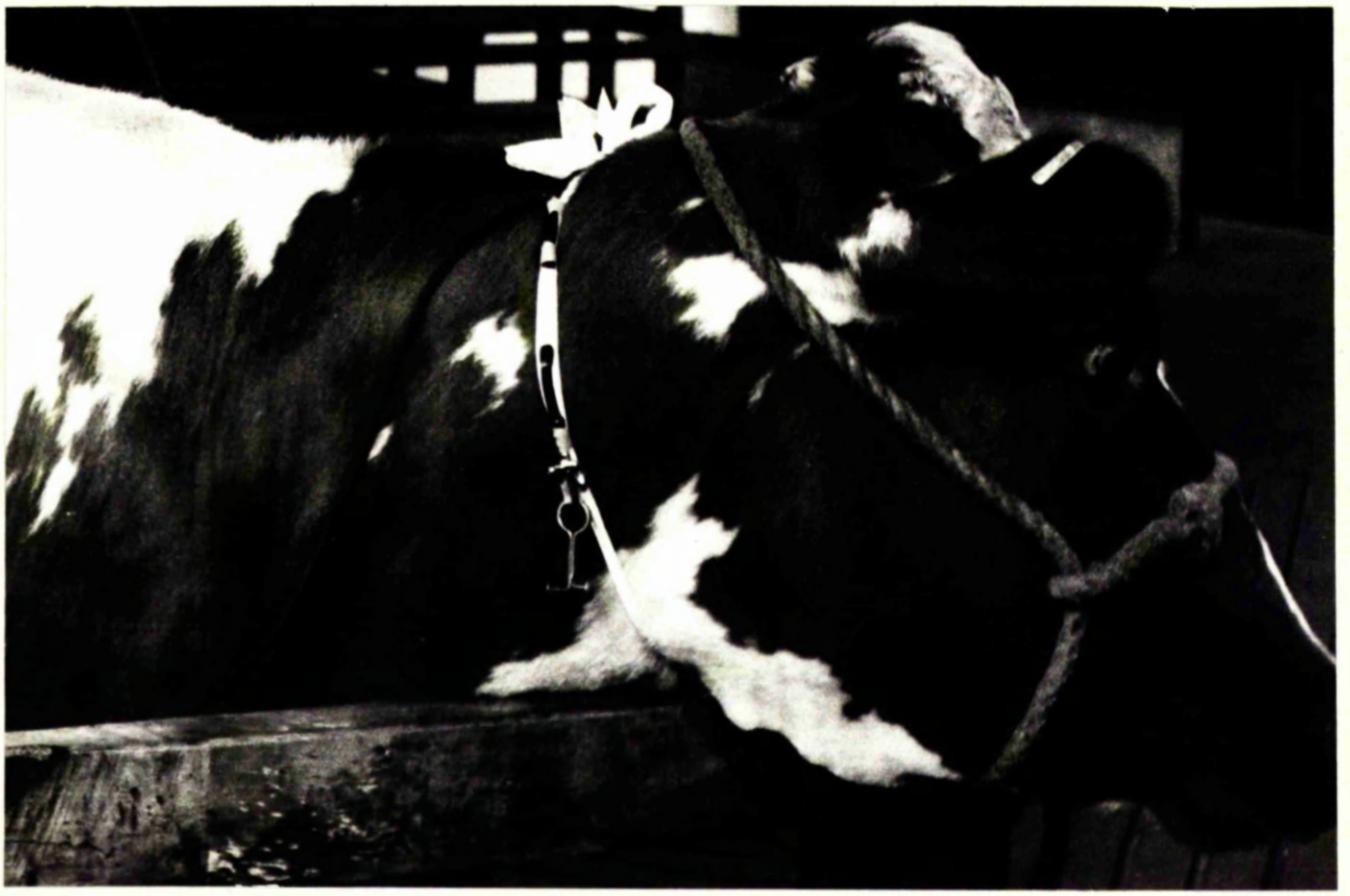


Fig. 14. Close-up of jugular cather *in situ*.

100mg/Kg. The solution was injected through the jugular catheter by gravity feed from a 500 ml. burette, and the experiment timed from the middle of the injection period.

To provide the necessary data for calculation of inulin clearance, blood and urine samples were collected at the following times:-

Blood

1. Before inulin injection - plasma blank (PB mg/100ml.)
2. 27 min after injection - P1 (inulin concentration mg/100ml. plasma)
3. 97 min after injection - P2 (inulin concentration mg/100ml. plasma)

Samples were collected in heparinised centrifuge tubes and spun down immediately after collection. There was a 3 min delay between blood sampling and the end of each urine collection period to allow for urinary delay time (p. 74).

Urine

1. Before inulin injection - urine blank (UB) mg/100ml.
2. 30 min. after injection - 1st sample (U1) mg/100ml.
3. 100 min. after injection - 2nd sample (U2) mg/100ml.

The volume of urine collected after 30 and 100 min. was also noted.

From the inulin concentrations and urine volumes measured in these samples, the volumes of distribution

of inulin at 27 and 97 min. were calculated from the expression -

$$V = \frac{IB}{P}$$

where V = volume of distribution
IB = total amount of inulin in the body
P = concentration of inulin in plasma

Clearance values were calculated from the results as described (p. 98.).

In 5 experiments values for inulin clearance were determined immediately after the last urine collection at 100 min by use of the formula -

$$c = \frac{u \times v}{p}$$

where c = inulin clearance rate (ml./min)
u = inulin concentration in urine (mg/100ml.)
v = rate of urine flow (ml./min)
p = inulin concentration in plasma (mg/100ml.)

Urine collection was carried out over 15 min. and a blood sample was taken 3 min. before the mid-point of the collection period.

Inulin concentration in plasma and urine was measured by the method of Schreiner (1950) and in later experiments by that of Roe, Epstein & Goldstein (1949). The former method was found to be less accurate than that of Roe et al and was abandoned.

The method described by Roe depended on the reaction of an inulin hydrolysate with resorcinol, giving a red colour. Plasma protein was removed by

precipitation with Sodium hydroxide and zinc sulphate (Somogyi, 1930), and colour developed by heating the tubes containing the samples and reagents in a water bath at 80°C. Readings were taken on a spectrophotometer* with maximum transmission of light at a wavelength of 520 mμ. A calibration curve from standard inulin solutions, 1, 2 and 3mg/l. was found for each experiment. Colour intensity agreed with the Beer-Lambert Law. Plasma protein concentration was determined by the biuret method, using the biuret reagent described by Weichselbaum (1946).

RESULTS

Inulin Space

Ten experiments on five cows were carried out using the method described. In each experiment the inulin space was found at 27 min. (V_1) and at 97 min. (V_2) after inulin injection. The results obtained are shown in Table 15.

The mean inulin space at 27 min. (V_1) was 69 ± 15 l., and at 97 min. (V_2) was 125 ± 34 l. V_2 was greater than V_1 in every experiment. Fig 15 shows the increase in the volume of distribution of inulin

* Unicam, S.P. 600

Table 15

Volume of distribution of inulin at 27 and 97 min in 5 cows.

Case no.	Weight (kg) *	Volume of distribution 27 min (V_1) (l.)	Volume of distribution 97 min (V_2) (l.)	V_1 %age body weight
10045	408	92	153	23
		91	137	22
10986	407	66	89	16
		63	109	15
9480/4	413	73	128	18
1122/237	501	72	111	14
		71	148	14
12401	419	70	115	16
		56	78	13
		59	129	14
Mean	430	69 ± 15	125 ± 34	16 ± 3

* Mean weight over experimental period.

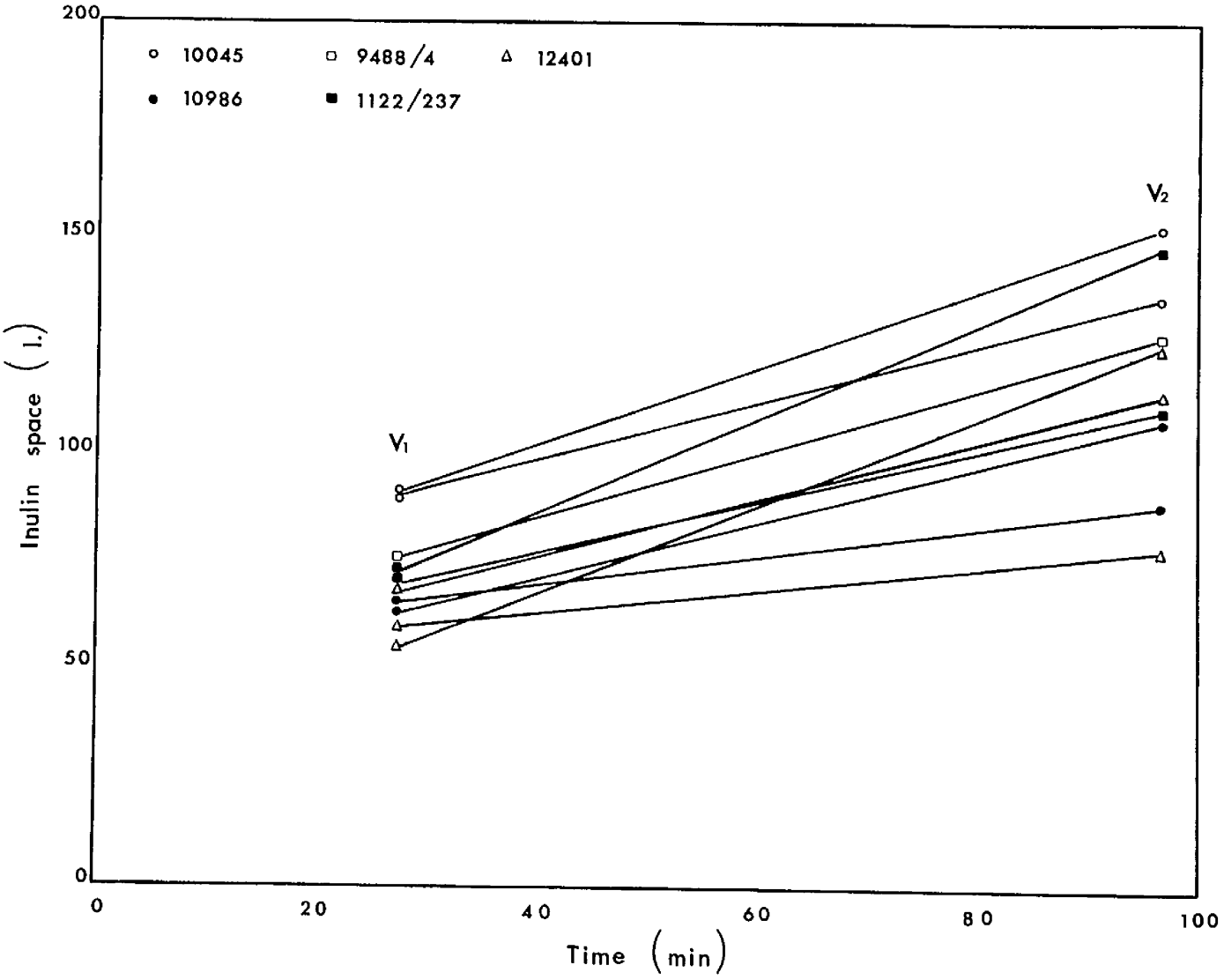


Fig. 15. Inulin space values in 5 cows measured at 27 min. and 97 min. after a single injection of inulin (100 mg/kg in 10% w/v solution in sterile normal saline).

between 27 and 97 min. after injection. The increase in inulin space which occurred in the 70 minutes between the two space estimations indicated that, equilibration of inulin distribution between plasma and extravascular fluid did not occur, and consequently V, the inulin space did not reach a constant value during the time of the experiment. The last column of Table 15 shows the inulin space at 27 min. (V_1) expressed as a percentage of the body weight in each experiment.

The clearance of inulin from plasma water was calculated from this data, and converted to units of ml. of plasma as described.

The results and calculation of a single representative experiment are shown on the following pages.

Results and calculation of an experiment to determine the glomerular filtration rate of a cow after a single injection of inulin

Case number:- 12401

Weight:- 419Kg

Inulin Concentrations and Amounts

Urine and Solution for injection

Sample	Inulin (mg/100ml.)	Volume (ml.)	Amount of inulin contained (mg)
Urine			
At 30 min	2360	560	13200
At 100 min	1800	735	13200
Injection	7600	500	38000

Plasma

Sample	Inulin (mg/100ml. plasma)	Inulin (mg/100ml. plasma water*)
At 27 min(P1)	33.0	35.5
At 97 min(P2)	9.4	10.1

* Inulin concentration in plasma water was calculated by multiplying the plasma concentration by the factor

$$\frac{100}{100\text{-g protein/100ml.}}$$ (Coldring & Chasis, 1944)

Calculation

Total inulin in the body (IB) = amount injected − amount excreted.

At 27 min. IB = (38000 - 13200) mg

= 24800 mg

At 97 min. IB = (38000 - 26400) mg

= 11600 mg

$$V_1 = \frac{IB}{p}$$

$$= \frac{24800}{35.5}$$

= 699 decilitres*

$$V_2 = \frac{11600}{10.1}$$

= 1148 decilitres

$$b = \frac{V_2 - V_1}{t_2 - t_1}$$

$$= \frac{1148 - 699}{97 - 27}$$

$$= \frac{449}{70}$$

= 6.4

$$C = \frac{b(\log p_1 - \log p_2)}{\log V_2 - \log V_1} - b$$

$$= \frac{6.4\ (\log 35.5 - \log 10.1)}{\log 1148 - \log 699} - 6.4$$

* Volumes of distribution are expressed in decilitres for convenience of calculation of clearance values.

$$= \frac{6.4 \times 0.5459}{0.1255} - 6.4$$

$$= 9.5 \text{ decilitres plasma water/min}$$

$$\text{Clearance} = \frac{9.5 \times 10,000}{93.1} \text{ ml. plasma/min.}$$

$$= \underline{1020 \text{ ml. plasma/min.}}$$

The results of the 10 experiments are shown in Table 16. In the last column of the table, results obtained by applying the conventional formula $\frac{u \times v}{p}$ are shown. In 10045 and 10986, there is good correlation between values obtained by the two methods, but in the other three animals there was no agreement between the two methods. Estimates of glomerular filtration rate using the single injection technique did not give repeatable results in the individual animal.

The mean glomerular filtration rate found by the single injection method was 998 ± 414 ml./min (range 446 - 1635 ml./min). The level of administration of inulin in the ten experiments was 98 ± 11 mg/kg.

Side effects

In one experiment, a marked reaction to the injection of about 450 ml. of 10% inulin solution was observed. During the injection, acute dyspnoea, tachypnoea and coughing developed. This abnormal respiration improved during the experiment, and returned to normal after 2 hrs. There was no pyrexia. A similar experiment was carried out on this cow seven days later without any side effects. The reaction was attributed to pulmonary oedema resulting from rapid intravenous injection of fluid (Elkington & Danowski,

Table 16

Inulin clearance values in 5 cows.

Case no.	Weight (Kg)	Inulin dose rate (mg/Kg)	Plasma Clearance Formula 1* (ml/min)		Plasma Clearance Formula 2* (ml/min)
				(Mean)	
10045	408	109	446	(530)	566
		84	613		
10986	407	109	1131	(1333)	1328
		91	1535		
9488/4	413	114	1635		
1122/237	501	84	1372	(1000)	1488
		100	627		338,308
12401	419	91	1020	(873)	549
		93	714		
		104	875		
Mean ± S.D.	430 ± 40	98 ± 11	998 ± 414		762 ± 512

* Formulae 1 and 2 shown on Table 16 (a).

Table 16 (a)

Formula 1.

$$C = \frac{b(\log P1 - \log P2)}{\log V2 - \log V1}$$

$$\text{Clearance} = \frac{C \times 10,000}{100 - \text{g plasma protein/100 ml}} \text{ ml/min}$$

Formula 2.

$$\text{Clearance} = \frac{U \times V}{p} \text{ ml/min}$$

1955).

In the course of this work, continuous efforts were made to establish a reliable technique for measuring urine flow in cattle. When such a technique was found, the main reason for use of the method described above was removed, and accordingly no further experiments using the single injection technique were attempted.

DISCUSSION

Failure to establish a reliable method of measuring urine flow in cows over periods of 15 to 30 min. led to use of the technique described. It was hoped that this method would prove useful in routine measurements of renal function in cattle without the necessity of setting up continuous infusion apparatus, and that such advantages would out-weigh the criticisms of single injection techniques in the measurement of renal function (Smith, 1951).

The results showed that in experiments using cows, an adaptation of the single injection technique used by Robson *et al* (1949) in man did not give reliable results. Despite the unreliability of the absolute

values of the clearances measured, some information was derived from the results.

The values obtained for inulin space at 27 min. after a single intravenous injection of inulin were 16 ± 3% of body weight.

The only other reports of inulin space measurement in cattle are those of Retz (1960) who found a mean value of 10.3% of body weight, and Anderson & Mixner (1960). Using a single injection technique on one cow Anderson & Mixner found that the inulin space was 15.3% of body weight. The method used by these workers depended on their finding that inulin reached equilibrium in the body after 45 min. The results of the present study, that in all cases the apparent inulin space rose between 27 and 97 min, agrees with the findings of Robson et al (1949) who showed that at no time between 16 and 150 min. after inulin injection was a stable value for inulin space obtained. It is concluded that, in the cow, as in man, equilibrium between plasma and extravascular inulin concentrations was not achieved. The minimum equilibration time in the dog is 1 to 2 hrs, and in man is 6 hrs. (Bunim, Smith & Smith, 1937; Gaudino, Schwartz & Levitt, 1948). In the opinion of these workers and Smith (1951), a single injection technique is, therefore, unsuitable

for measurement of extracellular space in man.

The above evidence and the present work indicates that there is little justification for use of a single injection technique as described by Anderson & Mixner, (1960), for measurement of extracellular space in cattle. For the same reasons, calculation of renal clearance of inulin made by these workers on the assumption of equilibrium between plasma, and extravascular inulin cannot be valid.

The mean value for inulin clearance in cattle obtained by the method described in the present study was 998 ± 414 ml./min. Despite the large standard deviation, this mean value is similar to those obtained by other workers, and in subsequent work by the present author (p.123). It is clear, however, that the repeatability of measurements was poor. Much of the inaccuracy of this method was certainly due to incomplete collection of urine from the bladder. It may be that, with the improved techniques of urine collection used in later work (p. 80.) this method would have yielded results of greater accuracy.

The technique was not, however, suitable for renal function studies in which serial measurements of electrolyte clearances were required in conjunction with the filtration rate. Only a single determination of

filtration rate was possible and consequently its usefulness in this field of study was limited. Once an accurate and simple urine collection technique had been established in the cow therefore, this method ceased to provide a useful alternative to the continuous infusion and continuous collection technique.

SUMMARY

A single injection technique for measurement of inulin clearance in the cow is described. Continuous collection of urine was not necessary to the method.

The results of 10 experiments are recorded and discussed in relation to those of other workers using single injection methods.

It is concluded that the use of this method in the cow had several disadvantages, and no advantages, when compared with constant infusion and constant collection methods.

Section 4

Measurement of inulin clearance by a constant infusion method

Measurement of inulin clearance by a constant infusion method

While the single injection method of measuring inulin clearance has advantages in the clinical investigation of renal function, the constant infusion method is preferred in studies of renal physiology.

A constant plasma level, as obtained by the infusion method, obviates many of the difficulties implicit in a single injection method. The uncertainty of reaching equilibrium between plasma and extracellular fluid, and the difficulty of relating a falling plasma concentration to the rate of excretion are avoided. The main source of error in a constant infusion technique is failure to measure urine flow accurately. Winton (1956) stated that under good steady state conditions, renal clearance measurements involve uncertainties of 5 to 10 per cent, and Smith (1951) ascribed these uncertainties to the difficulty of obtaining complete bladder drainage.

A brief outline of urine collection methods used in renal function studies and a detailed description of the technique developed and used in the present work is given in Part I, section 2(e).

The method developed and used for determining glomerular filtration rate by constant infusion of

inulin in cattle is described below, and the results of experiments using this method are recorded.

METHOD

The characteristics of inulin which make it suitable for use in the measurement of glomerular filtration rate in man are well known (Smith, 1951), and evidence for its suitability for renal function studies in the cow has been presented (p. 97.).

Clearance may be defined as the volume of plasma which is required to supply the amount of a substance which appears in the urine in unit time. When the clearance substance is completely filterable at the glomerulus and is neither reabsorbed, excreted, nor metabolised in the renal tubules, then the clearance rate is equal to the glomerular filtration rate. Inulin fulfills these requirements.

Inulin clearance is calculated from the formula:

$$\frac{u \times v}{p}$$

where u = concentration of inulin in the urine

v = the rate of urine flow

p = concentration of inulin in the plasma

The calculated value obtained from this formula is taken to be a measure of the glomerular filtration rate and is expressed as ml./min.

Experimental determinations of renal clearance of inulin were carried out on seven non-pregnant, non-lactating Ayrshire cows under the conditions of management and restraint described (p. 16).

The jugular veins and bladder were catheterised as described (p.102), blood and urine samples were taken for inulin blank estimations and preliminary urine collection made. During this period, the infusion apparatus was set up. Inulin* for infusion was dissolved in sterile NaCl. solution (0.9g/100 ml.) to a concentration of 5g/100 ml. A priming dose of this solution (10 ml./50kg body weight) was injected intravenously, and infusion begun by gravity feed from a 2 l. aspirator bottle through a Murphy drip tube. Inulin infusion rate was controlled at 2.5 ml./min by adjustment of the outflow tap of the aspirator bottle. The rate of flow of drops in the Murphy tube was repeatedly checked, but further adjustment of the outflow tap was rarely necessary. In later experiments this infusion apparatus was replaced by a constant infusion pump+ which was calibrated to deliver 2.5 ml. per min. Fig. 16 shows a general view of the infusion apparatus.

After starting the inulin infusion, 30 mins were

* The British Drug Houses Ltd.

+ Micro-pump (Distillers Company Ltd.)

Fig. 16. Experimental animal in stocks with inulin infusion apparatus. Aspirator bottles with adjustable outflow taps, and Murphy drip tubes leading to the jugular catheter are shown.

allowed to elapse before commencing a clearance period to give time for the plasma inulin concentration and the excretion of inulin to stabilise. The renal appearance time, i.e. the length of time it takes the most rapidly moving particles of an injected substance to appear in the urine, is about 2 to 3 min (p.75), while the main portion of an excretary load following a single injection is somewhat more delayed, and may be taken to occur following a delay of approximately twice the appearance time. In initial studies it was found that plasma inulin concentration had stabilised in the cow within 15 mins. after the start of infusion, so it was assumed that the renal excretion of inulin had stabilised 30 mins. after the start of infusion. The repeatability of inulin clearance determinations helped to confirm this assumption.

Blood was sampled from the left jugular vein, each 20 ml. sample of blood being taken into a syringe which had been rinsed with heparinised saline. Samples were immediately run into 15 ml. centrifuge tubes containing 2 drops of Heparin, and spun to separate the plasma. During 30 min clearance periods, samples were taken at 0 and 30 min and the mean inulin concentration of these two samples was used in the clearance calculation. During 15 min clearance

periods, one sample only was taken at 7 min.

Continuous urine collection was established by urethral catheter as described (p. 80). Urine was collected in 250 ml. measuring cylinders and the volume noted at the end of each clearance period. In early experiments, residual urine in the bladder was washed out with three separate washings of 100 ml. of water or saline at the end of each period, but latterly this procedure was stopped as it was found to be unnecessary to the accuracy of the technique. After each collection period, urine was transferred to polythene bottle, stoppered and labelled.

Plasma and urine samples were diluted with distilled water to give an estimated inulin concentration of 1 to 3 mg/100ml. Plasma was diluted 1:10 and urine between 1:250 and 1:1,000, depending on the rate of urine flow. Inulin concentration was measured by the method described by Roe et al (1949), using standards of 1, 2 and 3 mg/100ml. for each series of estimations.

Each experiment occupied a period of two days. On the first day, four to six 15 to 30 min clearance measurements were made, blood samples were centrifuged and the plasma separated, and urine samples were diluted. The dilution of urine samples reduced the

risk of inulin coming out of solution at high urinary concentrations. On the second day, chemical analysis of inulin and electrolyte concentrations were carried out, and clearance values calculated.

RESULTS

Accuracy of inulin estimations

The following results were obtained from an experiment to show the accuracy of the method of inulin estimation. Table 17 shows the results of 10 determinations carried out on a plasma sample to which inulin was added to give a concentration of 15 mg/100ml. The mean inulin concentration found in the 10 samples was 14.4 ± 0.4 (S.E.± 0.1) mg/100ml. plasma. Having measured the colour in each tube directly after colour development, the samples were allowed to stand on the bench for 30 mins. and the optical density again measured. The mean inulin concentration found on this second reading was 15.1± 0.6 mg/100ml. (S.E. ± 0.2). This mean value was not significantly different from that found 30 min. previously, but in 7 out of 10 samples, the optical density had increased.

The solution of inulin in plasma which was used in these experiments was prepared 72 hours before the inulin estimations and allowed to stand in a stoppered

Table 17

Results of duplicate determinations on a bulk sample of plasma to which inulin had been added to give a concentration of 15 mg/100 ml.

Sample	Inulin Immediate reading (mg/100 ml)	Inulin 30 min delay (mg/100 ml)
1	14.4	15.0
2	14.4	15.6
3	14.4	14.4
4	14.4	14.4
5	15.0	15.0
6	14.4	15.6
7	14.4	15.0
8	15.0	16.4
9	14.4	15.0
10	13.6	14.4
Mean ± S.D.	14.4 ± 0.4	15.1 ± 0.6
S.E.	± 0.1	± 0.2

100 ml. flask over this period. The close agreement between the expected and the determined inulin concentrations indicated that inulin solution in plasma remains fairly stable at room temperature for several days.

As inulin is a polymer consisting principally of fructose molecules (which are reabsorbed by the renal tubules), samples of the inulin solution infused were analysed for the presence of free fructose. Using the method of Somogyi (1952), it was found that fructose was not present in measurable amounts (< 1%).

In five experiments, the arterio-venous difference in inulin concentration was determined. Venous blood was obtained from the jugular catheter, and arterial blood by puncture of the coccygeal artery. The inulin concentrations found in these samples is shown in Table 18. As it was not possible to take the venous and arterial samples simultaneously, the time relationship between the samples in three experiments is shown in Fig. 17.

The values found in a typical experiment to measure inulin clearance are shown in Table 19. In this and other early experiments, a blood sample was taken at the beginning, middle and end of each period to assess the errors arising from fluctuations in

Table 18

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

Inulin concentration	
Arterial blood [x] (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

[x] Arterial samples were taken from the coccygeal artery within 15 min of the venous samples.

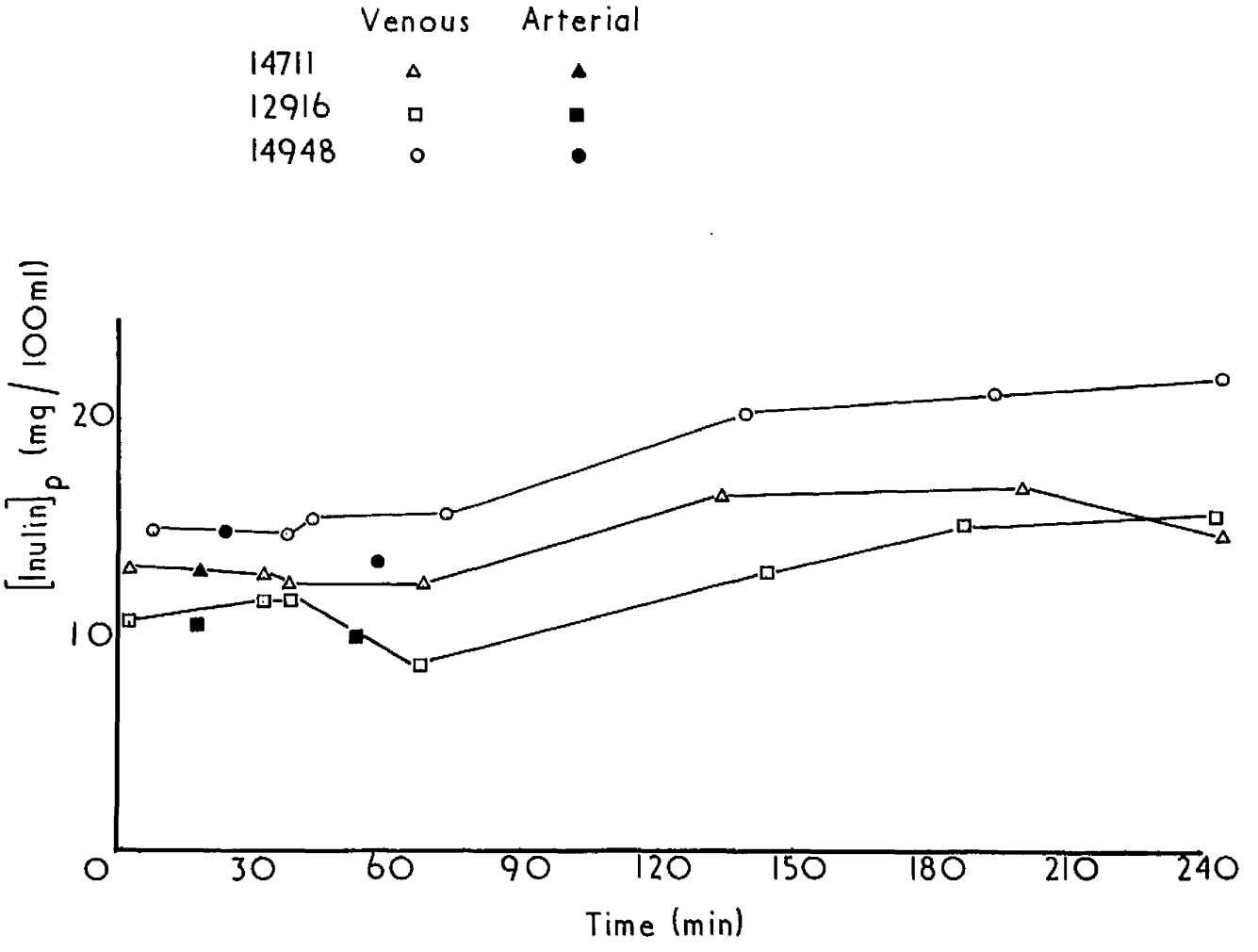


Fig. 17. Inulin concentrations in arterial and venous samples from 3 cows in the course of renal clearance measurements. Each arterial sample (closed symbols) was taken between two venous samples (open symbols).

Table 19

Case No. 12916 Weight 990 lbs. (450 Kg.)

Inulin Clearance Determination

Period	Time	Duration (min)	Urine flow (ml/min)	Urine Concn. (mg/100 ml)	Plasma concn[x] (mg/100 ml)	Clearance (ml/min)
1	2.20 - 2.53	33	20.8	542.0	10.0 9.4 (10.8) 10.0	1151
2	3.00 - 3.31	31	17.7	636.6	9.8 10.5 (10.0) 9.8	1128
3	3.40 - 4.10	30	18.4	636.2	10.5 10.0 (10.6) 11.2	1110
4	4.20 - 4.50	30	18.6	580.8	11.2 10.8 (10.9) 10.8	967

[x] In this experiment, blood samples were taken at the beginning, middle and end of each period.

The figures in brackets in column 6 are the mean values of the three concentrations for each period.

plasma level during each period. The mean of the three values found was used in calculation of the clearance rate. The results showed that little fluctuation in plasma level occurred during each period and so a single plasma sample in the middle of each clearance period was taken as being representative of the concentration during the period.

Table 20 shows 26 observations on one cow (14711) in six experiments extending over a period of 18 months. There was no constant trend for clearance values to rise or fall over this period, and most clearance values were within $\pm$ 10% of the mean value. Despite the absence of any general trend, however, it was found that the mean values on separate experimental days were significantly different from each other. For example, the mean clearance found on 1/8/60 (1031 $\pm$ 59 ml./min) was significantly higher than that found on 7/9/60 (898 $\pm$ 39 ml./min) ($P < .01$). The same findings were recorded in other cows. In a few experimental days clearance values decreased towards the end of the experiment, but these findings were not so consistent or frequent as to constitute a general trend. Tables 21 to 26 show the results of inulin clearance measurements on the other 6 cows.

The independence of inulin clearance and the rate

Table 20

Case No. 14711 Weight 547 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
1. 8.60	996 946 1068 1058 1087	1031 ± 59
7. 9.60	918 843 941 929 894 864	893 ± 39
10.11.60	1111 1256 846 956 998 1000	1028 ± 141
20. 7.60	892 893 1026 907 920	927 ± 56
Mean and S.D.	963 ± 95	965 ± 54

Table 21

Case No. 12916 Weight 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

Table 22

Case No. 14948 Weight 477 Kg.

Date	G.F.R. (ml/min)	Mean daily G.F.R. (ml/min)
6. 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 ± 43

Table 23

Case No. 1122/237 Weight 434 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

Table 24

Case No. 13107 Weight 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 25

Case No. 1122/315 Weight 401 Kg.

Date	G.F.R. (ml/min)	Mean daily G.F.R. (ml/min)
13. 7.59	1436	
	1321	
	1295	
	1119	1293 ± 132
15. 7.59	1103	
	1090	
	1103	
	989	1071 ± 55
Mean and S.D.	1182 ± 151	1182 ± 157

Table 26

Case No. 12401 Weight 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

of urine flow was shown in experiments in which diuresis was induced by infusion of $\frac{N}{4}$ KCl solution during a series of clearance measurements. A marked and well sustained diuresis resulted from this procedure, and the results of a typical experiment are shown in Fig. 18. After the first two clearance periods, KCl infusion was started and urine flow rose from 15 ml./min to 67 ml./min being an increase of 250%. At the same time the inulin clearance rose from 1199 ml./min to 1274 ml./min, being an increase of 6%.

In 30 experiments, 103 measurements of the renal clearance of inulin was made on 7 cows whose weights ranged from 401 to 547 Kg. The clearance values obtained are shown in Table 27.

The mean rate of inulin clearance varied from 838 $\pm$ 96 ml./min to 1234 $\pm$ 133 ml./min. The average of the seven mean values was 1005 $\pm$ 147 ml./min. When the mean value for each cow was expressed on a unit weight basis, the over-all mean for the seven cows was 1,100 $\pm$ 236 ml./min/500 Kg body weight. The increased standard deviation resulting from expressing these values on a unit weight basis indicated that in these animals, there was no relationship between glomerular filtration rate, and body weight.

On one occasion, an animal showed a reaction to

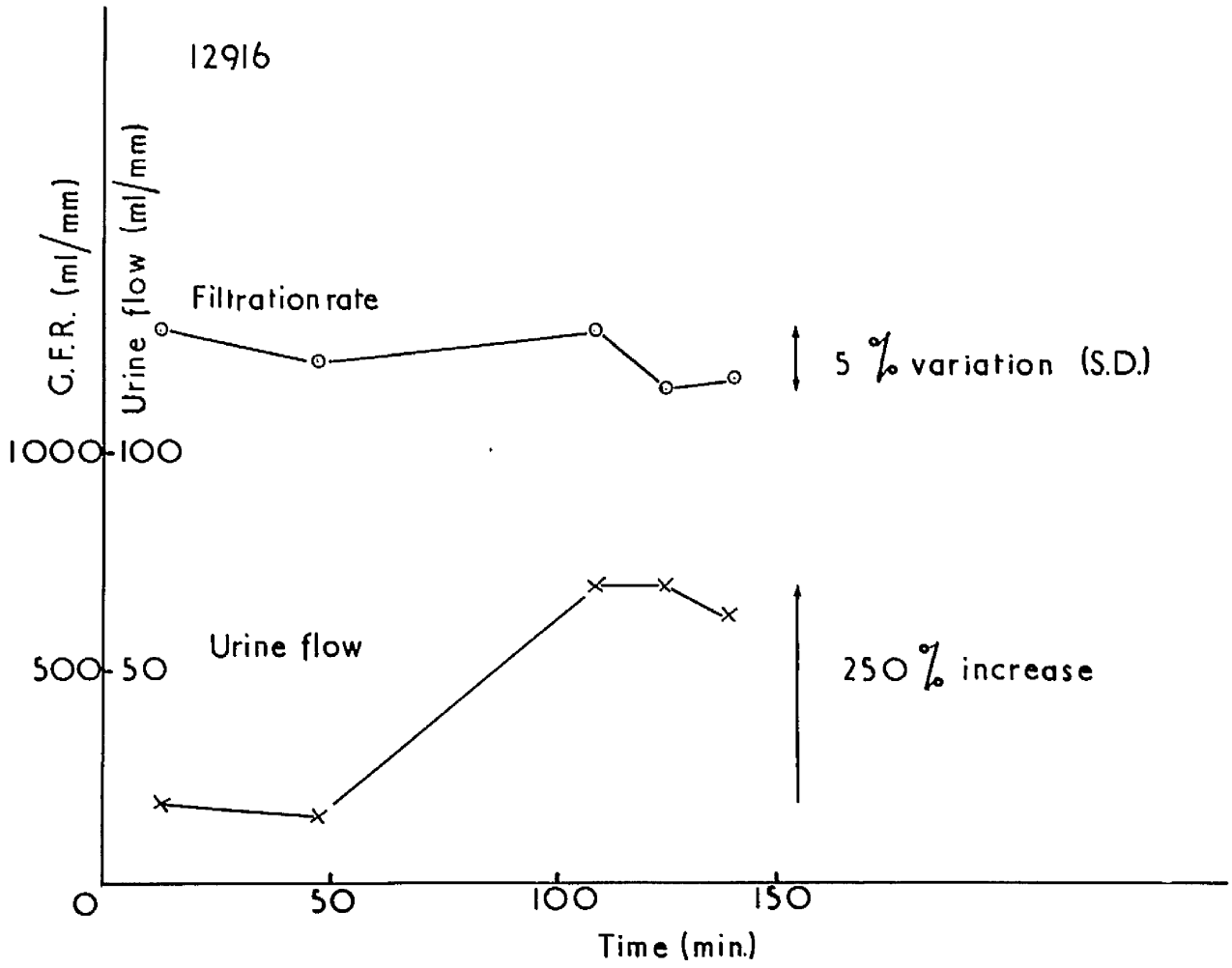


Fig. 18. Glomerular filtration rate during a diuresis caused by the intravenous infusion of $\frac{1}{4}$ KCl. solution.

Table 27

Case No.	Body Weight[x] 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

[x] Average weight over the period of experimentation.

the experimental technique described. After 60 min infusion with 2.5% inulin at 5 ml./min, tachypnoea, dyspnoea, and coughing developed. A venous blood sample taken at this time was noticeably darker in colour. The animal returned to normality within 2 hours of the onset of respiratory distress. On several occasions, transient coughing occurred in other animals during or after rapid intravenous injection of 100 ml. of 5% inulin into the jugular vein.

DISCUSSION

The clearance technique used in the present work was largely based on that described by Poulsen (1957, 1959), with a modified technique for the measurement of urine flow (Anderson & Pickering, 1961). It was found to give results which were as consistent as those obtained from dogs and man under optimal experimental conditions. The constant infusion technique allowed serial clearance measurements to be made, and was much more reliable than the single injection technique previously described.

In renal clearance work, there may be difficulty in separating true variations in clearance values from variations due to experimental error. The preliminary experiments described were designed to assess the magnitude of the experimental error.

In an experiment to test the repeatability of the chemical methods, and to assess the percentage recovery of inulin added to a plasma sample, the results indicated that -

1. A standard solution of inulin in plasma kept in a stoppered flask for 72 hours on the laboratory bench remained stable to within 0.6 mg/100 ml. of the expected concentration, giving a recovery rate of 96%.

2. Ten duplicate estimations on a single sample of plasma made up to contain an inulin concentration of 15 mg/100 ml. gave a mean value of 14.4 $\pm$ 0.4 (S.E. $\pm$ 0.1) mg/100 ml. plasma. Thus variations in the inulin clearance values due to the chemical techniques showed a standard error of less than 1% of the mean value and were not therefore important in a technique where the accuracy expected under optimum conditions was 5 - 10%.

3. It appeared that the colour developed did alter on standing on the bench as stated in the description of the method (Roe et al, 1949) but in the conditions of our experiment there was a tendency for the optical density to increase rather than diminish.

In testing for the presence of free fructose in the inulin used for injection, another possible source of error was examined. As inulin is a polymer made up largely of fructose molecules which are normally

reabsorbed by the renal tubules, it was important to show that there was no free fructose in the inulin used in the clearance experiments. The presence of a reabsorbable fraction of fructose in the injected inulin would have invalidated its use for measurement of glomerular filtration rate. It was shown that the inulin used contained an insignificant proportion of free fructose.

Agreement between inulin concentrations in arterial and venous blood as shown in Table 18 was anticipated but confirmed the justification for using venous blood samples as being representative of the arterial blood presented at the glomeruli. The assumption of equality of arterio-venous concentrations is not justifiable in a single injection method, as theoretically arterial blood has a lower inulin concentration than the systemic venous blood in such a method.

The infusion technique described maintained a fairly stable level of inulin concentration in the plasma, as shown in Table 19. Variations in clearance values which occurred could not, therefore, be attributed to fluctuations in plasma concentrations.

In the absence of significant errors arising from these sources, 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 the glomerular filtration rate. Urine collection errors will always contribute to variability of results in a technique such as that described as there is inevitably a small residuum of urine in the bladder at the end of each collection period. Variability in actual rates of glomerular filtration are well recognised particularly under experimental conditions (Deyrup 1947; Davies & Shock 1950; Winton 1950; Wolf 1950; Smith 1951) and there is some evidence in the present work to indicate true day to day variations in glomerular filtration rate in the cow.

The significant variations in day to day clearance values which were shown in Table 20 were also evident in experimental records in the thesis of Poulsen (1956). In the present work there was, however, no trend to diminishing values towards the end of a series of measurements on one experimental day such as was described by Wolf (1950) and Smith (1951). McDonald & Macfarlane (1958) found that the glomerular filtration rates of sheep were higher in a hot than in a cold environment, but in the present study, there were no variations in inulin clearance which were significantly related to seasonal changes.

It was shown in Fig. 17 that a marked elevation of the rate of urine flow resulted in an insignificant alteration in glomerular filtration rate. This confirmed Poulsen's (1957) findings that inulin clearance rate in the cow is not altered during diuresis.

Table 28 shows the mean values of inulin clearance found in the present study, and by other workers. The results of Sellers et al (1958) and Kotz (1960) were expressed in the original text as ml./sq m surface area. To allow comparison with other work, these values have been converted to ml./500 Kg body weight by the general formula relating surface area to body weight:

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

As the original authors did not make it clear which formula was used in deriving surface area, the figures shown in Table 22 can only be approximate.

The present work was undertaken to establish a reliable technique for measuring the rate of filtration, reabsorption and excretion of electrolytes by the bovine kidney. The results indicate that the method used is comparable in reliability to experiments carried out on man and dogs under carefully controlled laboratory conditions. Examination of sources of experimental error and of the results of serial clearance measurements on individual animals on various

Table 28

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[x] (894 - 1360
Ketz (1960)	10	16		858[x] ± 156
Anderson (1964)	7	30	103	1100 ± 236 (878 - 1474

[x] These values were expressed in the original articles in ml/min/unit of surface area.

The general formula

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

was used by the present author to relate these values to body weight.

days, suggest that experimental error is not generally responsible for the variations in filtration rate recorded, and that true variations in filtration rate were measured by this technique.

SUMMARY

The technique used in measurement of glomerular filtration rate in the cow by a continuous infusion of inulin solution is described.

Sources of experimental error are examined and the results recorded.

Evidence is presented for true day to day variability in glomerular filtration rate in cattle.

The results of 103 measurements of glomerular filtration rate in 7 cows are recorded.

The findings are discussed and compared with those of other workers.

P A R T IX

A STUDY OF THE RENAL EXCRETION OF BICARBONATE IN COWS

INTRODUCTION

Previous work on bicarbonate excretion has been carried out on species in which the normal diet has an acid ash and the individual is faced with necessity to excrete the acid end-products of metabolism without becoming depleted of sodium. The present extensive knowledge of tubular acidification by ionic exchange mechanisms and ammonia secretion is the result of this work. An understanding of the renal regulation of bicarbonate excretion and reabsorption has played an integral part in the development of present concepts.

The first critical examination of the renal regulation of bicarbonate excretion was by R.F. Pitts (Pitts & Lotspiech, 1946) and since then this worker has been responsible for much of the present knowledge of the renal control of acid-base balance. He showed that in man, if plasma bicarbonate concentration were lowered by ingestion of NH_4Cl, all the bicarbonate filtered through the glomeruli was reabsorbed, and none was excreted until the plasma level was subsequently elevated to a value of 26-28 mM/l. - which he referred to as 'the so-called renal bicarbonate threshhold'. As the plasma concentration increased above 28 mM/l, a limited amount of bicarbonate, equal

to 2.8 mM per 100 ml. of glomerular filtrate was reabsorbed. Bicarbonate reabsorption in the dog was essentially the same except that the threshhold was slightly lower (24-26 mM/L.) and the transport rate slightly less (2.6 mM/100 ml. of glomerular filtrate). In the same work, he found that in samples of alkaline urine, the curvilinear relationship between the measured pH and bicarbonate concentrations gave calculated values of pCO_2 greatly in excess of those of plasma. This was contrary to previous tenets (Gamble, 1922) who stated that over a wide pH range, urine pCO_2 remained relatively constant at the same level as in plasma.

More recently, important contributions to the understanding of bicarbonate reabsorption have been made by Rector and Seldin and their co-workers (Portwood, Seldin, Rector & Cade, 1959; Rector, Seldin, Roberts & Smith, 1960; Rector & Seldin, 1962), and within the last year Clapp and co-workers have developed and applied micropuncture studies of bicarbonate reabsorption in the proximal convoluted tubule of the dog (Clapp, Watson & Berliner, 1963a, b). Pitts (1963) has collated much of the recent work and has pointed out that the renal threshhold for bicarbonate reabsorption is by no means an invarying constant

and is influenced by at least four factors: (1) the pCO_2 of arterial blood, (2) the plasma level of chloride (3) the body store of potassium (4) secretion of adrenal cortical hormones.

Bicarbonate reabsorption is regarded as the result of tubular secretion of H^+ ion in exchange for Na^+, (Pitts & Alexander, 1945). An adequate supply of H^+ ions is ensured by hydration of CO_2 to carbonic acid in the tubular cells, this reaction being catalysed by carbonic anhydrase. (Fig. 19).

It has been shown that two of the principal determinants of H^+ secretion in the renal tubules are the CO_2 tension of plasma (Dorman, Sullivan & Pitts, 1954), and the activity of the carbonic anhydrase enzyme system (Pitts & Alexander, 1945).

In a series of carefully designed experiments on dogs, Rector et al (1960) concluded that bicarbonate reabsorption was a result of two distinct processes. One process, assigned to the proximal tubule, had a bicarbonate tubular maximum dependent on plasma CO_2 tension, and independent of carbonic anhydrase. A second process, apparently located in the distal tubule, had a bicarbonate tubular maximum which was dependent on carbonic anhydrase, and independent of changes in plasma pCO_2.

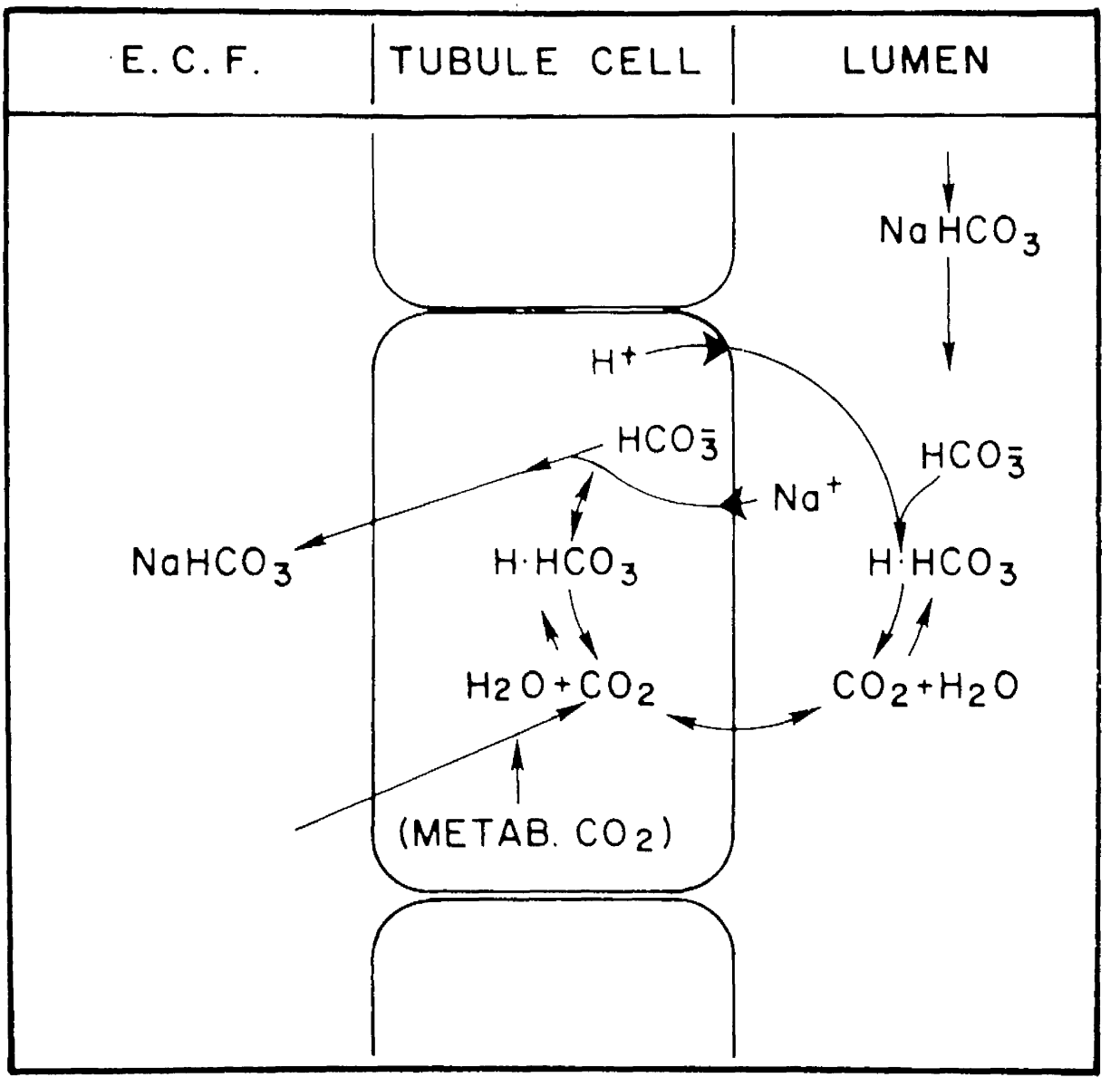


Fig. 19. Schematic representation of the role of the hydration of CO_2 and $H^+ - Na^+$ exchange in the reabsorption of sodium bicarbonate (Bayer & Baer, 1961).

Recent micropuncture studies (Clapp et al, 1963b) have, however, shown that carbonic anhydrase is important to the reabsorption of bicarbonate from the proximal tubular fluid in rats. Thus it seems likely that the H^+ and Na^+ exchange mechanism, which is responsible for bicarbonate reabsorption, takes place in proximal and distal portions of the nephron, and that at both sites it is dependent on the availability of carbonic anhydrase and variations in plasma pCO_2.

There is no information on the renal control of bicarbonate excretion in herbivores. It is known that cattle excrete alkaline urine (Dukes, 1955), and Fisher (1959) and Aalund (1960) reported that the normal level of bicarbonate in bovine arterial plasma is about the same as that of man. If these findings were correct, then no other conclusion could be drawn than that renal regulation of bicarbonate excretion in the cow was different from that of man.

The alkalinity of the herbivores urine is usually attributed to the excess of inorganic cations in the diet (Cushny, 1926; Dukes, 1955; Pitts, 1963). Robinson (1961) stated that alkalinising salts such as potassium citrate and sodium lactate, as well as vegetable diets which contain large amounts of potassium balanced by organic anions reduce the net amount

of acid formed in the body, and may cause the urine to become alkaline. The organic anions which are absorbed are apparently metabolised, leaving bicarbonate behind to accompany the cations, so that the ingestion and subsequent metabolism of these salts is tantamount to the ingestion of bicarbonate. It has been known for some time that the oral administration of potassium chloride resulted in the excretion of alkaline urine (Loeb, Atchley, Richards, Benedict & Driscoll, 1932; Bourdillon, 1937), and there have been numerous studies of the inter-relationships of potassium metabolism and the distribution and excretion of bicarbonate (Berliner, Kennedy & Hilton, 1950; Roberts, Magida & Pitts, 1953; Fuller, McLeod & Pitts, 1955). These studies have usually been carried out on dogs, and potassium has either been administered orally as a potassium salt, or intravenously. There is, however, little experimental evidence to support the widely held belief that a vegetable diet causes alkaline urine. Hunt (1955) cited Bodansky (1938) as stating that 'most vegetables and fruits .... are base forming and yield an alkaline urine'. The acid - or base - forming characteristics of the diet referred to the acidity or alkalinity of the ash of the diet which was calculated from the difference between the content of Na^+, K^+,

Mg^{++}, and Ca^{++} on the one hand, and Cl^{-}, $P^{=}$ and $S^{=}$ on the other. If the inorganic cations predominated, as they do in most vegetable matter, the food was said to have an alkaline ash. Despite keeping his human subjects on a diet of alkaline ash for 10 - 14 days Hunt rarely found urinary pH greater than 7.0, even though two subjects were 'Vegans' of several years standing. He found that the acidity of the urine was rather related to the sulphur content of the diet, than to the acidity or alkalinity of its ash.

There is no doubt that the diet of the cow is of vegetable matter, and rich in potassium (Morrison, 1951) and that it excretes an alkaline urine (Dukes, 1955), but there is surprisingly little evidence to establish a causal relationship between those two facts. Smith (1956) stated that the excretion of an acid or an alkaline urine depends on the fate of sodium bicarbonate in the tubules, and Pitts (1963) has recently stressed the central role of the renal regulation of bicarbonate excretions in acid-base balance. As a first step therefore in examining the part played by the kidney in the maintenance of acid-base balance in the cow, the renal excretion of bicarbonate was examined.

Carbonic anhydrase plays an integral part in the

acidification of urine in man and the dog, its primary importance being in maintaining the supply of hydrogen ions for exchange with sodium ions in the tubular fluid. The administration of carbonic anhydrase inhibitors such as acetazolamide cause a marked reduction in the availability of hydrogen ions, with a consequent elevation of urinary pH, natriuresis increase in bicarbonate excretion, and diuresis. (Berliner & Orloff, 1956).

It has been shown that the effect of potassium infusion in dogs caused a similar increase bicarbonate excretion and elevation in urinary pH as occurred after administration of acetazolamide, and that the effects of these two agents were not additive during simultaneous administration (Fuller, McLeod & Pitts, 1955). Thus dogs receiving an infusion of acetazolamide showed little or no response to a superimposed infusion of potassium bicarbonate.

Cattle normally excrete an alkaline urine and are consequently less dependent on the urinary acidification mechanisms of which carbonic anhydrase is an integral part. They are, furthermore, on a high potassium intake under normal management conditions. As both these factors are relevant to the action and effect of a carbonic anhydrase inhibition, it was of interest to

examine the effect of acetazolamide in the cow.

Hydrochlorothiazide, a heterocyclic sulphonamide, which, unlike acetazolamide, does not inhibit carbonic anhydrase at therapeutic dosages, had been widely used in cattle for the relief of clinical oedema (Cowie, 1960; Fluckiger & Hofer, 1960; Johnston, 1961; Vigue, 1961). The effect of this drug on electrolyte excretion in cattle was examined under the same experimental conditions as acetazolamide.

Section 1

A survey of pH and bicarbonate concentrations in urine samples from a herd of dairy cows

A survey of pH and bicarbonate concentrations in urine samples from a herd of dairy cows

It is generally accepted that under most conditions of management bovine urine is alkaline. The pH values quoted in standard works of reference (Dukes, 1955; Sipple, 1963) and by most investigators (Ashworth & Brody, 1933; Galloway, 1936; Szolnocki, 1941; Poulsen, 1957; Barrada, 1957) are usually between 7.0 and 8.4. With the exception of Szolnocki, however, these results were based on urine samples drawn without anaerobic precautions, and indeed some were obtained from aliquots of 24 hr samples collected in a car-boy and mixed thoroughly before sampling. It has long been known that human urine samples rarely exceed a pH of 8.0, but if exposed to air, lose CO_2 and assume an artificially elevated pH (Marshall, 1922). As the previously reported pH values for cattle were probably all elevated by exposure to air, an initial survey of normal pH and bicarbonate values in urine of healthy dairy cattle was undertaken. The effect of exposure to air on the pH and bicarbonate values has been reported elsewhere (Part I, Section 1).

METHOD

Samples were obtained from a herd of 36 Ayrshire dairy cows under indoor conditions of management in February and March. All animals were on the same basic diet of 40 lbs silage, 8 - 10 lbs hay, and a supplement of $4\frac{1}{2}$ lbs cattle cake per gallon of milk. A further series of samples was taken from 30 of the same animals at grass in May of the same year. Again a supplement of $4\frac{1}{2}$ lbs of cattle cake per gallon of milk was fed. A record was kept of the stage of pregnancy and/or lactation of each animal sampled. All urine samples were obtained by catheterisation, and the urine collected under oil. The collection, transport, storage, and the methods of measuring total CO_2 and pH have been described elsewhere (Part I, Section 1). The pH values obtained at room temperature were converted to $39^{\circ}C$ by use of a Temperature - pH correction factor of 0.0053 pH units per $^{\circ}C$ (Wesson, 1953). Bicarbonate concentration was calculated from the Henderson - Hasselbach equation assuming values for $pK' = 6.1$ and $\alpha = 0.0309$.

Thus: $$pH = pK' + \log\frac{(\text{Total } CO_2) - \alpha pCO_2}{\alpha pCO_2}$$

It was appreciated that values for pK' and α vary slightly from sample to sample due to variations in

the total ionic concentration of the urine. Recent work has, however, shown that the calculated values for urine pCO_2 and bicarbonate obtained by this method differ by less than 1% from the values actually measured (Kain, Carrasquer, Shapiro & Brodsky, 1957). In later experiments where sodium and potassium concentrations were measured, pK' was calculated for each sample according to the formula

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

where D is the total cation concentration of the sample.

As sodium and potassium constitute at least 95% of the cation in the bovine urine, the sum of these cations may be taken to equal the total cation concentration (Hastings & Sendroy, 1925).

Values for pK' were also calculated from the nomogram published by Severinghaus, Stupfel & Bradley, (1956), but results were found to be almost identical to those calculated by the above method.

RESULTS

Under the conditions described all the urine samples were alkaline and contained appreciable quantities of bicarbonate.

In the winter, while under indoor conditions of

management urine samples from 36 cows had a mean pH of 7.97 ± 0.22 (Range 7.32 - 8.21) and a mean bicarbonate concentration of 137.5 ± 56.5 mM/l. (Range 26.5 - 219.1).

In the spring, when at grass, urine samples from 30 cows had a mean pH of 7.87 ± 0.25 (Range 7.36 - 8.20) and a mean bicarbonate concentration of 123.9 ± 60.6 mM/l. (Range 20.0 - 254.4). The results are shown in Table 29.

There was no significant difference between the pH and bicarbonate concentrations as a result of the change from indoor to outdoor conditions, indeed the values were very similar.

There was no significant difference between pregnant and non-pregnant groups, nor between lactating and non-lactating groups.

In individual animals, there was no relationship between the bicarbonate and pH values of urine sampled in February or March and that sampled in May.

The mean pH of the 66 samples was 7.92 ± 0.21 (Range 7.32 - 8.21) and the mean bicarbonate concentration was 131.0 ± 58.3 mM/l. (Range 20.0 - 254.4).

The relationship between urinary bicarbonate concentration and pH is shown in Fig. 20. In addition to the 66 observations already described, a further 22 samples obtained from clearance experiments are included in the graph. As these samples were obtained

Table 29

The bicarbonate concentrations and $pH^{39^{\circ}C}$ values of urine samples from a herd of Ayrshire dairy cows under indoor and outdoor conditions of management.

Time of year	Management	No. of cows	HCO_3 (m/l)	Range	$pH^{39^{\circ}C}$	Range
Feb-March	Indoor	36	137.5 ± 56.5	26.5 - 219.1	7.97 ± 0.22	7.32 - 8.21
May	Outdoor	30	123.9 ± 60.6	20.0 - 254.4	7.87 ± 0.25	7.36 - 8.20
Feb. March and May	Indoor and Outdoor	66	131.0 ± 58.3	20.0 - 254.4	7.92 ± 0.21	7.32 - 8.21

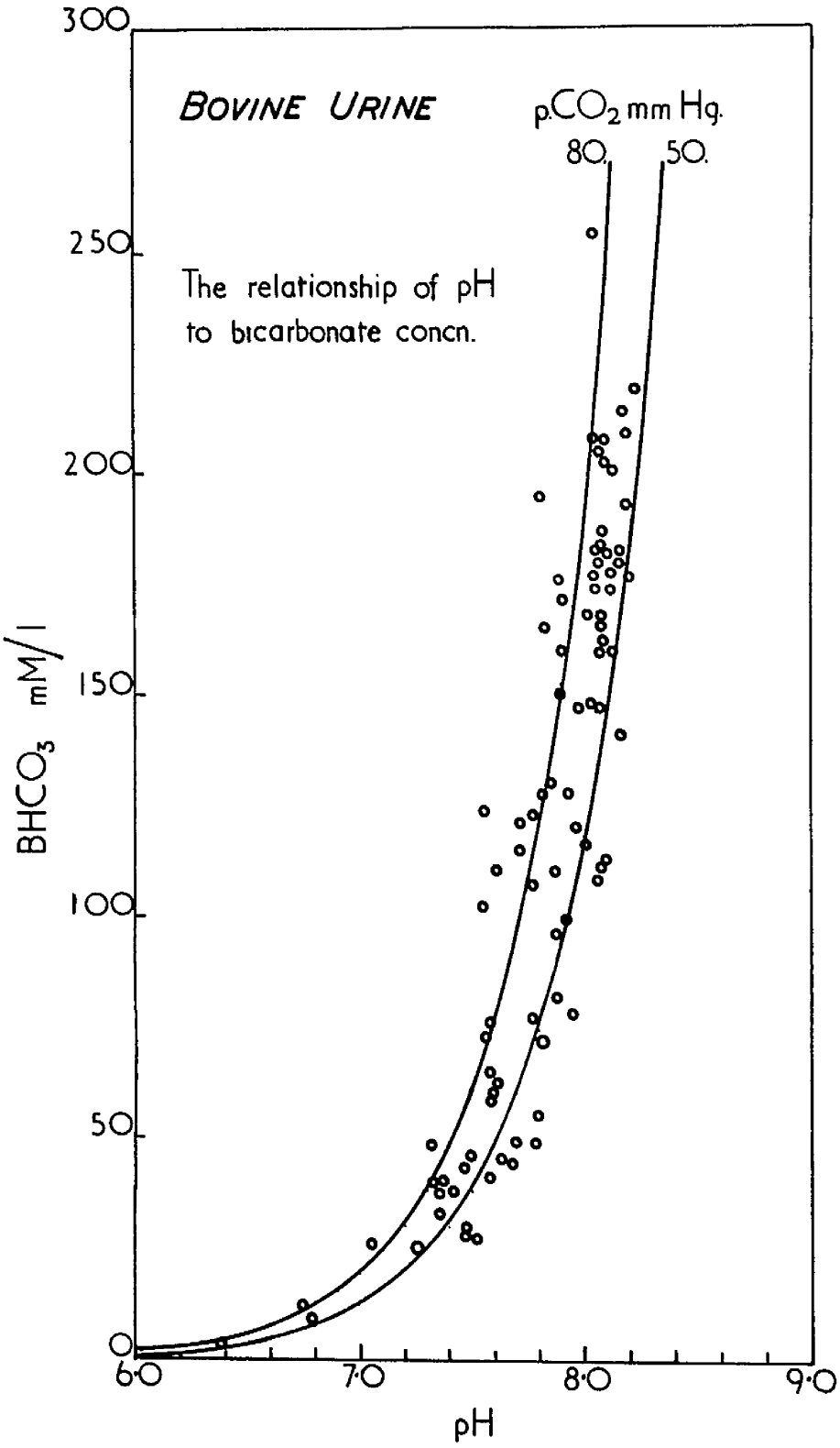


Fig. 20. The relationship between pH and bicarbonate concentration in 88 samples of urine collected anaerobically from Ayrshire dairy cows. The curved lines represent the theoretical relationship between pH and bicarbonate concentration when pCO_2 = 50 and 80 mm Hg.

from animals kept under a different management regime, they were not included in the mean values recorded in the previous paragraphs.

The two smooth curves on this graph represent the theoretical relationship of pH to bicarbonate concentration at CO_2 partial pressures of 50 and 80 mm Hg. These were calculated from the Henderson-Hasselbach equation, assuming pK' = 6.1, and α = 0.0309 (Sendroy, Seelig & Van Slyke, 1934). Of the 88 points marked, 58 (two thirds of the total) lie within the pCO_2 range of 50 to 80 mm Hg delineated by the two curves. The samples with a high bicarbonate concentration exceed a pCO_2 of 80 mm Hg, and the samples with a low bicarbonate concentration have a pCO_2 of less than 50 mm Hg. The mean pCO_2 calculated for the 88 samples was 64 $\pm$ 22 mm Hg. (Range 26 - 159).

At high pH and bicarbonate concentrations, the total CO_2 in urine samples exists largely as bicarbonate (Peters & Van Slyke, 1932). Thus, in analysis of normal bovine urine samples, only a small error is incurred when total CO_2 concentrations are assumed to be equivalent to bicarbonate concentrations. For example, using the mean values found in the present study, it was found that 98.5% of the total CO_2 existed as bicarbonate. (Table 30).

Table 30

Calculated value of %age total CO_2 existing as bicarbonate in 66 samples of bovine urine.

Mean total CO_2 (measured) (mM/l)	Mean pH (measured)	Mean bicarbonate (calculated)[x] (mM/l)
133.0	7.92	131.0

$$\%age\ total\ CO_2\ as\ bicarbonate = \frac{131}{133} \times 100$$

$$= \underline{98.5\%}$$

[x] Calculation as described in text.

Section 2

Normal variations in bicarbonate excretion and their relationship to plasma bicarbonate concentration in 12 cows

Normal variations in bicarbonate excretion and their relationship to plasma bicarbonate concentration in 12 cows.

In the following experiments, total CO_2 of urine and plasma and the pH of urine were measured by the method described. Blood pH was measured in some experiments, but the performance of the glass electrode was too unreliable to provide useful results. Comparison of the total CO_2 concentration of urine and arterial blood was, however useful, as it had been shown in the previous section that, at the normal pH values of bovine urine, the total CO_2 concentration is 98.5% bicarbonate, and in subsequent experiments (p.153.) it was found that there was little variation in the dissolved CO_2 component of the total CO_2 concentration of arterial plasma. Urine collection was by a Foley balloon catheter (Maine 20FG, 100 ml. bulb capacity with a modified end piece (Fig. 22), using the techniques described (p. 80.). Arterial blood was obtained by puncture of the brachial artery using the method described by Fisher (1956). The correct site was located as described, and after clipping, swabbing, and anaesthetising the skin, a 4 inch x 18 gauge needle was introduced into the brachial artery. When a free-

flowing pulsatile stream of arterial blood was obtained, an extension of 4 in. of rubber or polythene tubing was fitted to the hilt of the needle. Blood was run under 5 ml. of neutralised liquid paraffin in a heparinised 50 ml. MSE centrifuge tube. The tube was sealed with a rubber bung displacing most of the paraffin and centrifuged at 20°C to separate the plasma.

Twelve Ayrshire cows kept under indoor management and on a diet of hay and supplementary concentrates were used in these experiments. As different stages of pregnancy and lactation caused no significant difference in urinary total CO_2 concentration (p.141.) or in arterial plasma total CO_2 concentration (Fisher, 1959), no attempt was made in the following work to ensure that all the cows were non-pregnant, as this would have limited the number of subjects available. A record of their reproductive histories was, however, kept, and again the results were not affected by pregnancy.

RESULTS

In 60 experiments on the 12 cows total CO_2 concentration of arterial plasma was measured and compared with the urinary excretion of total CO_2. In early experiments, arterial plasma was sampled during

each clearance period, but as the total CO_2 values obtained showed little variation, (Table 31) it was decided that the disturbance of repeated arterial punctures was likely to cause more unphysiological results than the error entailed in assuming a constant plasma level of total CO_2 during an experiment of about two hours. Subsequently, the value obtained from a single plasma sample was assumed to be a constant value for that experiment. Total CO_2 concentrations of plasma taken from the same cow on different days also showed some constancy, so that it was found that a particular animal had a characteristic concentration. Table 31 shows that case number 9488/1 had a consistently lower concentration that that of S328/B11.

The mean total CO_2 concentration in the plasma of the 12 cows examined was 29.1 $\pm$ 2.2 mM/L. (Range 26.2 - 33.0) Table 32.

As the mean concentration of dissolved CO_2 of arterial plasma calculated in subsequent experiments was 1.3 $\pm$ 0.1 mM/L, the approximate bicarbonate concentrations corresponding to these total CO_2 values was 27.8 $\pm$ 2.2 (24.3 - 32.4) mM/L. Over this range of plasma bicarbonate levels, the mean concentration of total CO_2 in the urine was 113.7 $\pm$ 51.8 mM/L. (Range 0.05 - 4.28). These values are shown in relation to

Table 31

Total CO_2 concentrations of repeat samples of arterial plasma from two cows.

Case No.	Date	Time	Total CO_2 (mM/l)
S328/B11	15. 3.57	10.00	31.0
		10.30	31.2
		11.00	31.2
		11.30	30.0
	17. 3.57	10.15	33.7
		10.45	33.7
9488/1	29.10.57	11.00	26.4
		11.45	27.4
	3. 2.58	10.00	25.6
		11.00	25.6

Table 32

Results of 60 measurements of the urinary excretion of total CO_2 and their relationship to total CO_2 concentration in arterial plasma in 12 cows.

Case No.	No. of Experiments	Mean (total CO_2) (plasma) (mM/l)	Mean (total CO_2) (urine) (mM/l)	Mean urine flow (ml/min)	Mean excretion rate (mM/min)
S328/B11	6	31.8	80.0	29.3	2.35
5369/R95	4	26.9	96.5	10.9	1.05
9488/1	12	26.4	111.4	11.2	1.25
9488/3	6	23.2	176.5	6.6	1.17
9488/4	10	28.2	119.2	17.8	2.12
10045	8	33.0	130.2	5.5	0.71
10160	8	29.5	93.7	7.6	0.71
9400	2	29.8	225.5	9.5	2.15
10150[x]	1	27.6	20.3	2.6	0.05
9627	1	31.0	80.6	12.0	0.97
10237	1	26.2	102.6	19.7	2.02
1122/92	1	30.3	128.0	15.8	2.02
12 cows	60	29.1 ± 2.2	113.7 ± 51.8	12.4 ± 7.3	1.38 ± 0.73

[x] This animal calved the previous day and developed acetonaemia on the day following the experiment.

the plasma concentrations of total CO_2 on Fig. 21. It can be seen that under the conditions of these experiments there was no relationship between plasma concentration, and the rate of excretion of total CO_2. The figure shows that when plasma total CO_2 concentration was as low as 25.6 mM/l. (24.3 mM/l. of bicarbonate) urine was alkaline and total CO_2 (98.5% bicarbonate) was being excreted by the kidneys.

It has been noted (p. 99.) that a marked diuresis frequently followed the stress of puncture of the brachial artery. While this was an undesirable side effect in the examination of renal function, it allowed study of the effect of diuresis from the stress on the excretion of bicarbonate and variation of pH. Figs. 22 to 24 show the results of three experiments in which there was a diuretic response to arterial puncture.

In Fig. 22 there was initially a sharp anti-diuresis during which urinary total CO_2 concentration increased. This increase in concentration was not, however, enough to prevent the over-all fall in bicarbonate excretion which occurred. There followed a diuresis which lowered total CO_2 concentration and thus caused little alteration in total output, and latterly, urine flow decreased, total CO_2 concentration increased, and output went up. Thus urine flow had

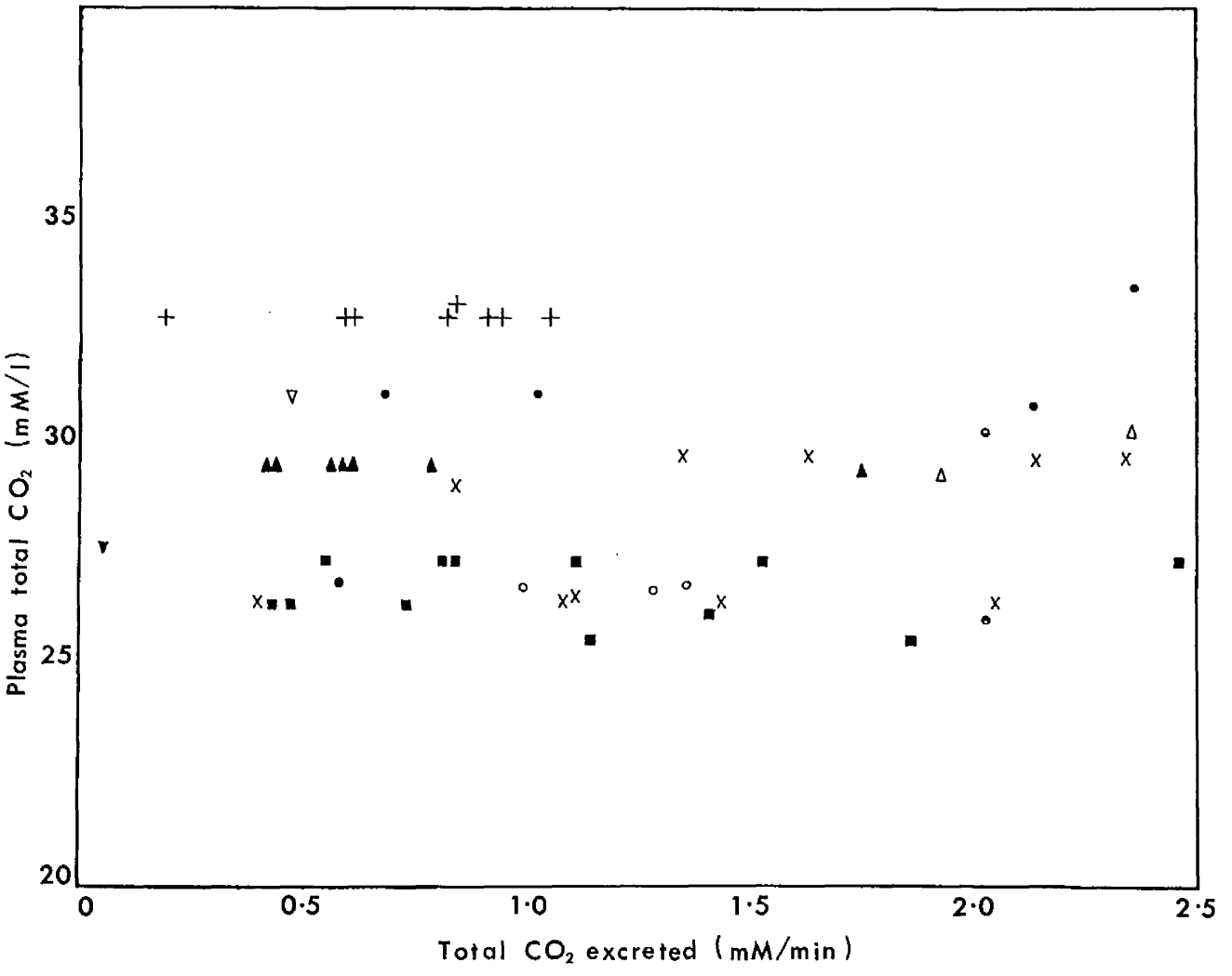


Fig. 21. Relationship between the rate of total CO_2 excretion and the total CO_2 concentration of arterial plasma sampled during urine collection.

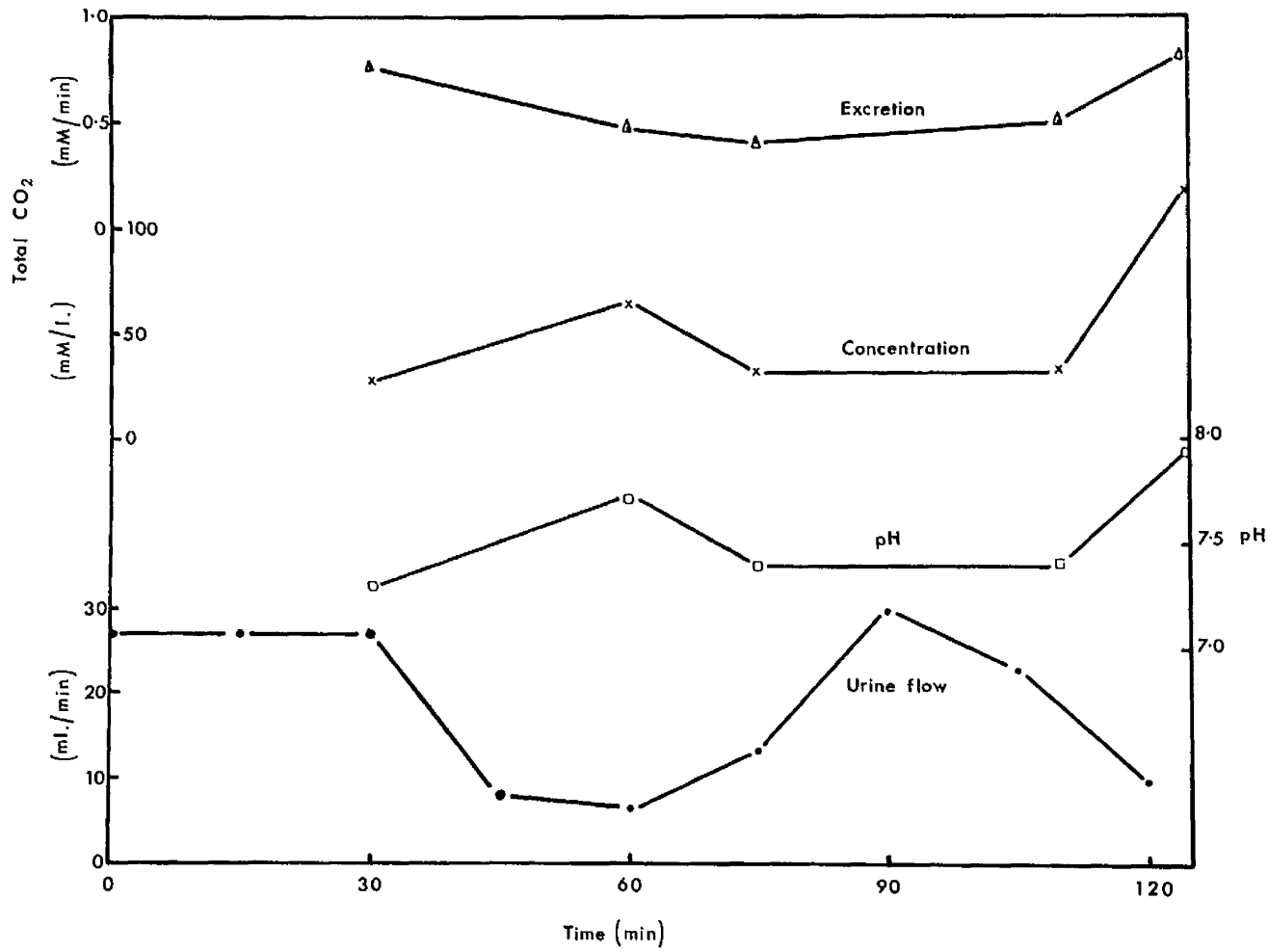


Fig. 22. Variations in urine flow, pH, and total CO_2 concentration and excretion during continuous urine collection.

a reciprocal relationship with total CO_2 concentration which tended to minimise changes in total CO_2 output. Output was however, increased in the last part of the experiment by a marked increase in urinary total CO_2 concentration despite falling urine flow.

The next experiment (Fig. 23) shows a sharp diuresis which, though accompanied by a fall in total CO_2 concentration, resulted in a marked increase in total CO_2 excretion. A third type of excretion pattern is illustrated in Fig. 24 in which there was a sudden rise in total CO_2 concentration causing an increased output, while the rate of urine flow remains constant. The results of these, and other experiments showed that while the concentration was reduced by any sharp increase in urine flow, thus tending to minimise the effect of urine flow on total CO_2 excretion rate, marked variations in excretion rate occurred as a result of variations in urine concentration during constant urine flow. While there was a reciprocal relationship between urine flow and urinary total CO_2 concentration in the individual experiment, there was no over-all correlation between urine flow and total concentration.

The effect of increase in urine flow on pH of urine was as expected from the relationship between bicarbonate and pH values found in the previous work.

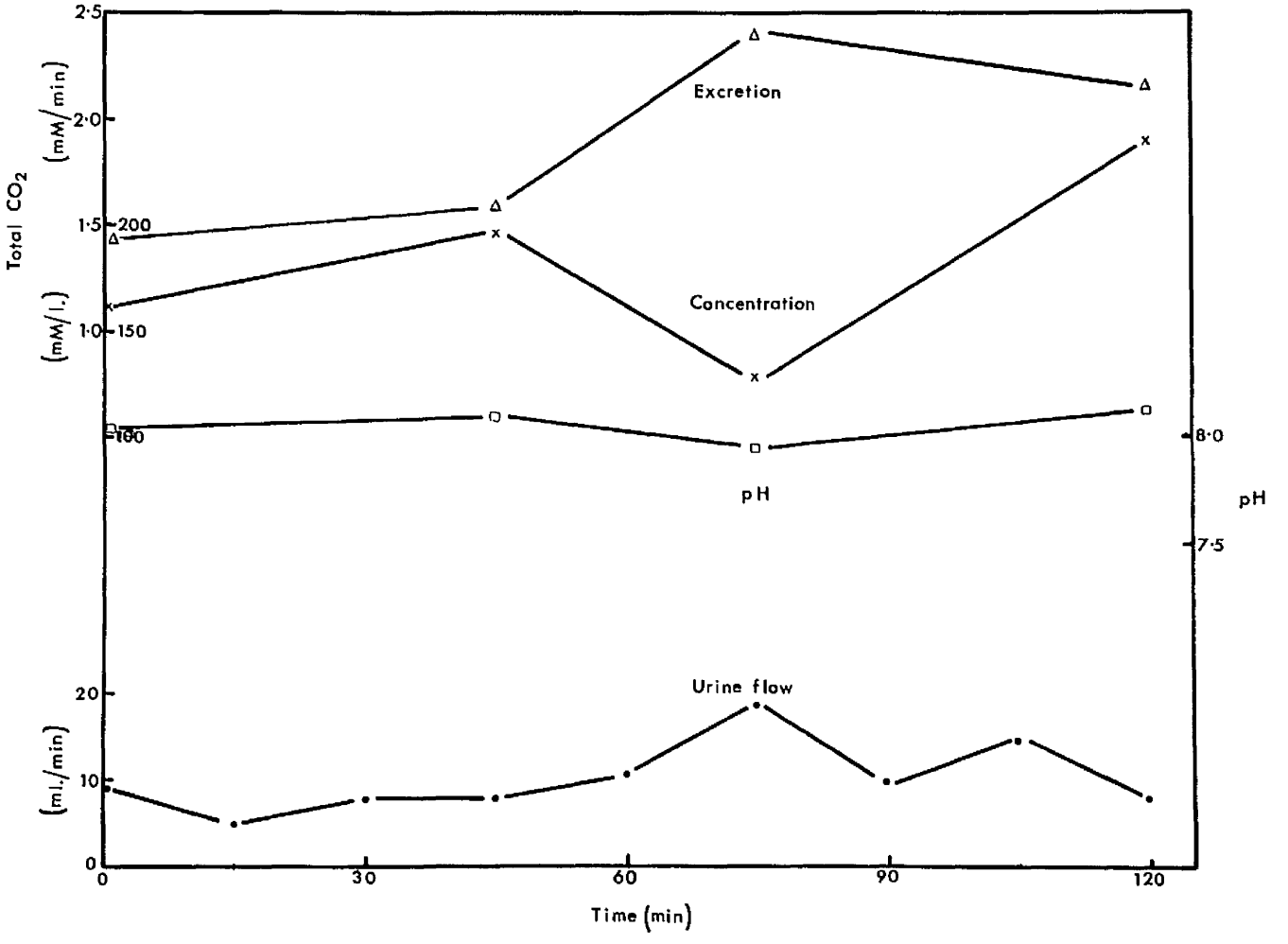


Fig. 23. Variations in urine flow, pH, and total CO_2 concentration and excretion during continuous urine collection.

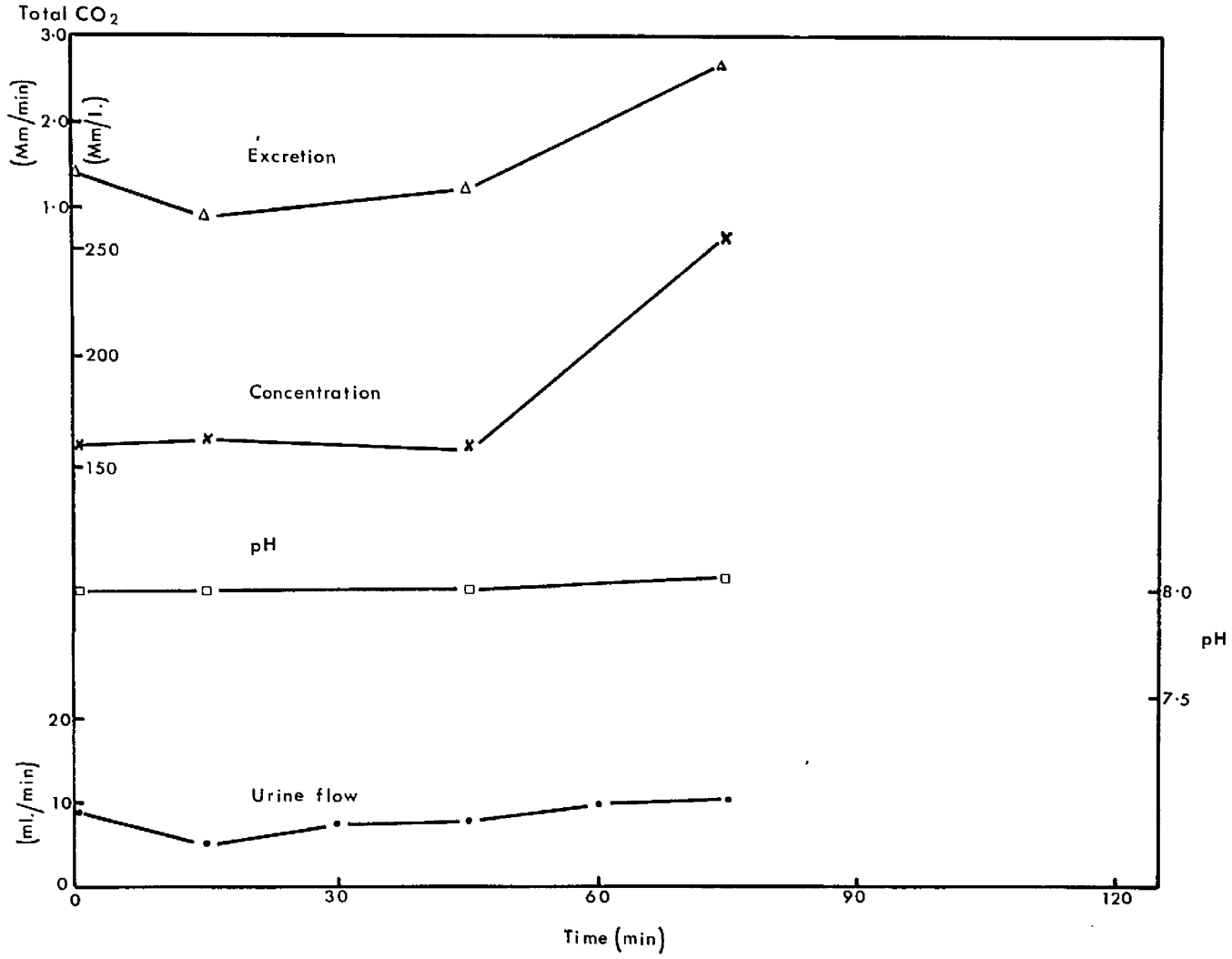


Fig. 24. Variations in urine flow, pH, and total CO_2 concentration and excretion during continuous urine collection.

If increased rate of flow resulted in a fall in total CO_2 concentration, there was a corresponding fall in urinary pH. In accordance with the pH - bicarbonate inter-relationship (p.141.) the change in pH induced by diuresis was greatest at low total CO_2 concentrations, and least when total CO_2 concentrations were high. Fig. 22 shows the effect of a diuresis on pH when the initial total CO_2 concentration was low (40 mM/l.). During the period of antidiuresis when urine flow fell from 27 ml./min to 7 ml./min, pH rose by 0.45 of a unit from 7.30 to 7.75, and during the subsequent diuresis pH fell to 7.45. In Fig.23 in which the urinary total CO_2 concentration was high the effect of dilution of the total CO_2 concentration was much less; during an increase in flow rate from 8.0 at 45 min to 19.0 at 75 min, pH fell from 8.01 to 7.95 - a fall of 0.06 unit - despite a sharp fall in total CO_2 concentration. A similar trend was shown in other experiments in which the urinary pH was measured. In these experiments, therefore, the pH-bicarbonate relationship found conformed to the pattern obtained in the previous work from individual samples collected from a normal dairy herd.

Section 3

Filtration, reabsorption and excretion of bicarbonate in 3 cows

Filtration, reabsorption and excretion of bicarbonate in 3 cows

In order to examine the renal control of bicarbonate excretion in greater detail, glomerular filtration rate was measured by the technique of continuous inulin infusion described (p.116.) at the same time as urine and plasma was sampled for total CO_2 and pH estimations. In these experiments, arterial blood was sampled from the coccygeal artery by a 17 gauge x 1 inch needle into an oiled heparinised 100 ml. syringe. Up to 100 ml. of blood was sampled to allow several estimations of pH on whole blood, and total CO_2 on plasma. Sampling was carried out at approximately the mid-point of the 30-min clearance periods. Whole blood pH was measured at 39°C (p.29.) and total CO_2 as described (p.24.) Urine was collected by a Ramshorn catheter (p.80.) and a sample for pH and total CO_2 estimation taken into an oiled 20cc syringe for pH and total CO_2 analysis.

From the results obtained, the rates of filtration, reabsorption and excretion of bicarbonate were calculated.

Filtration rate of bicarbonate =

$$\frac{\text{GFR ml./min} \times \text{HCO}_3^- \text{ plasma mM/l.}}{1000} \text{ (mM/min)}$$

Excretion rate of bicarbonate =

$$\frac{\text{Urine flow ml./min} \times HCO_3^- \text{ urine mM/l.}}{1000} \text{ (mM/min)}$$

Reabsorption rate of bicarbonate =

(Filtration rate - excretion rate) mM/min

or $\frac{\text{Filtration rate - excretion rate}}{\text{GFR/100}}$ mM/100 ml. glomerular filtrate

The number of estimations which were possible on one day were limited to two for the following reasons. Difficulty was experienced in obtaining more than two samples of arterial blood from the coccygeal artery during one experiment. This was partly the result of the development of haematomata after several attempts at arterial puncture, and partly as a result of increasing difficulty of insertion of the needle into the artery on the third or subsequent occasion. As the pulse was no longer palpable under these circumstances, it is possible that spasm of vessel wall severely reduced the lumen of the artery. Several attempts were made to place a retention needle or a fine nylon catheter in the artery to facilitate sampling, but were not successful.

RESULTS

Twenty determinations of the rate of filtration

reabsorption and excretion of bicarbonate were made on 3 cows. The results of all experiments are shown in Tables 33 to 35.

During the period of the experiments, plasma bicarbonate concentrations remained fairly constant in each cow - each animal having a characteristic plasma concentration (29.4 ± 2.1, 27.2 ± 1.1 and 25.0 ± 1.8) mM/l. The bicarbonate reabsorption rates were calculated as described, and plotted against plasma bicarbonate concentrations. It was found that there was a significant proportional relationship between plasma concentration and reabsorption rate /100 ml. glomerular filtrate ($P<.01$). Thus the cow with a high plasma bicarbonate concentration had a significantly greater reabsorption rate than the cow with the low bicarbonate concentration. The regression of bicarbonate reabsorption /100 ml. glomerular filtrate on plasma concentration is shown on Fig. 25. When bicarbonate reabsorption was expressed in mM/min, there was no relationship between reabsorption rate and plasma concentration.

Reabsorption rate per min was significantly related to glomerular filtration rate (Fig.26). Again the regression is significant at the 1% value for F.

The mean rate of bicarbonate reabsorption in the

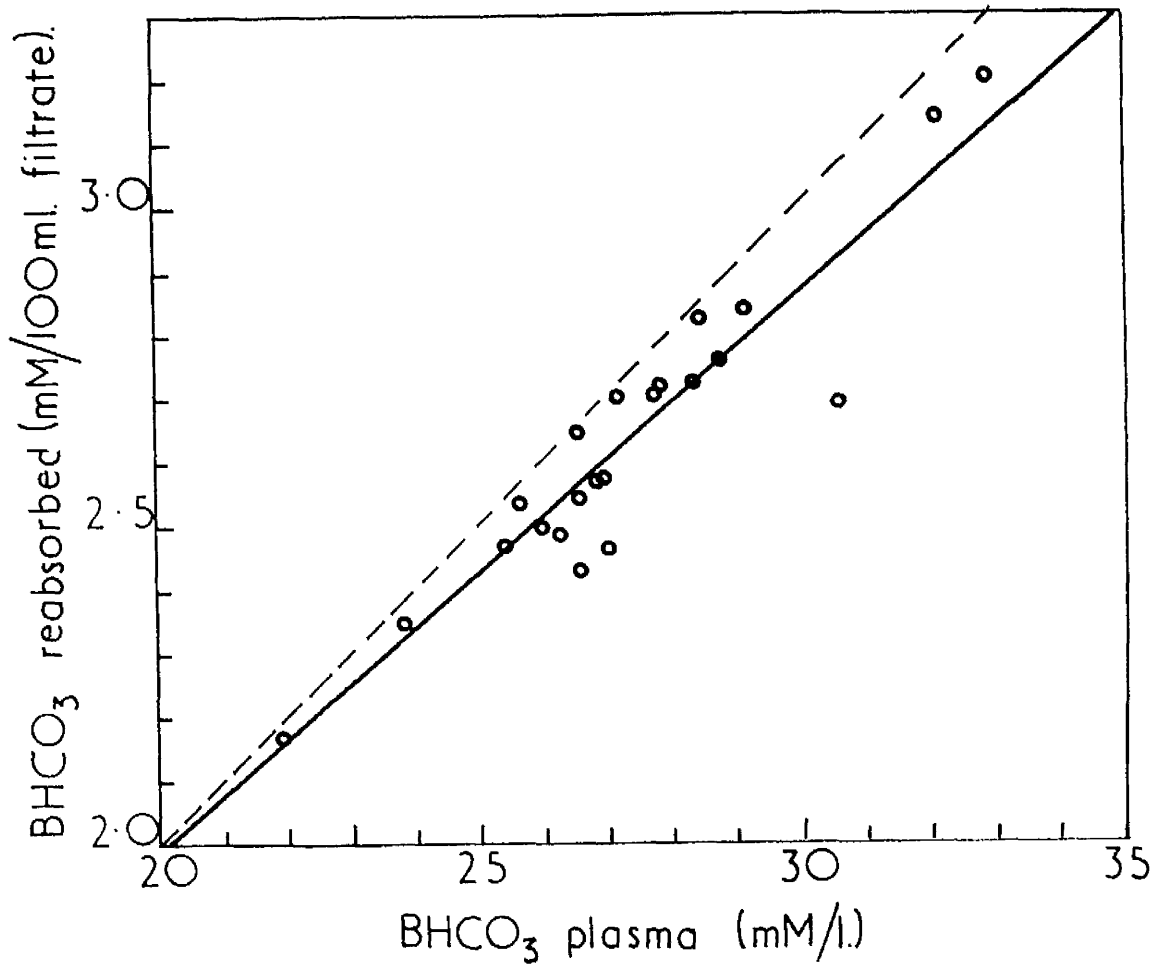


Fig. 25. The relationship of bicarbonate reabsorption rate to the concentration of bicarbonate in arterial plasma. The regression of the relationship shown by the continuous straight line is significant ($P<·01$). The interrupted line shows the relationship for 100% reabsorption.

Table 33

Collected results of 8 experiments to measure bicarbonate filtration, excretion and reabsorption in case number 12916.

Date	Wt. (Kg.)	G.F.R. (ml/min)	Arterial plasma pH	Arterial plasma HCO_3 (mM/l)	Arterial plasma pCO_2 (mmHg)	Urine flow (ml/min)	Urine pH	Urine HCO_3 (mM/l)	Urine pCO_2 (mmHg)	Bicarbonate Filt. (mM/min)	Bicarbonate Excr. (mM/min)	Bicarbonate Reabsd. (mM/min)	Per 100 ml. G.F. Excr. (mM)	Per 100 ml. G.F. Reabsd. (mM)
25. 7.60	522	1396	7.43	32.8	50	24.9	7.38	40.3	58	45.8	1.0	44.8	0.072	3.21
		1224	7.43	32.1	49	27.4	7.36	32.7	65	39.3	0.9	38.4	0.073	3.14
3. 8.60		1265	7.40	27.7	47	18.8	7.42	37.9	65	35.0	0.7	34.3	0.055	2.71
		1119	7.40	27.8	47	16.1	7.47	42.2	65	31.1	0.7	30.4	0.063	2.72
27.10.60		1250	7.49	29.1	40	18.0	6.74	11.1	94	36.4	0.2	36.2	0.016	2.90
		1250	7.50	30.5	40	16.9	7.05	25.4	100	38.1	0.4	37.7	0.016	3.02
17.11.60		1537	7.46	27.1	40	25.7	6.38	3.0	65	41.7	0.10	41.6	0.005	2.71
		1232	7.45	28.4	43	18.7	6.79	8.4	71	35.0	0.2	34.8	0.013	2.82
Mean S.D.	522	1284 ± 127	7.45 ± 0.03	29.4 ± 2.1	45 ± 4	20.8 ± 5.0	7.07 ± 0.40	25.0 ± 15.6	73 ± 16	37.9 ± 4.7	0.5 ± 0.3	37.3 ± 4.7	0.039 ± 0.029	2.90 ± 0.19

Table 34

Collected results of 6 experiments to measure bicarbonate filtration excretion and reabsorption in case number 14948.

Date	Wt. (Kg.)	G.F.R. (ml/min)	Plasma pH	Plasma HCO_3 (mM/l)	Plasma pCO_2 (mmHg)	Urine flow (ml/min)	Urine pH	Urine HCO_3 (mM/l)	Urine pCO_2 (mmHg)	Bicarbonate Filt. (mM/min)	Bicarbonate Excr. (mM/min)	Bicarbonate Reabsrd. (mM/min)	Per 100 ml G.F. Excr. (mM)	Per 100 ml G.F. Reabsrd. (mM)
8. 8.60	526	933	7.37	25.4	46	14.8	7.50	46.2	58	23.7	0.7	23.0	0.75	2.47
		973	7.41	26.0	43	16.9	7.62	61.8	58	25.3	1.0	24.3	0.103	2.50
20.10.60		1036	7.41	26.2	43	18.6	7.57	72.9	71	27.1	1.4	25.7	0.135	2.48
		894	7.41	26.5	43	12.5	7.82	164.5	81	23.7	2.0	21.7	0.223	2.43
24.11.60		897	7.38	21.9	43	5.3	7.38	41.1	58	19.6	0.2	19.4	0.022	2.16
		972	7.41	23.8	39	7.6	7.82	128.9	56	23.1	1.0	22.1	0.103	2.27
Mean	526	951	7.40	25.0	43	12.9	7.61	85.8	64	23.7	1.1	22.7	0.110	2.39
		±	±	±	±	±	±	±	±	±	±	±	±	±
S.D.		54	0.02	1.8	2	6.2	0.27	50.3	10	3.9	0.6	2.2	0.067	0.12

Table 35

Collected results of 6 experiments to measure bicarbonate filtration, excretion and reabsorption in case number 14711

Date	Wt. (Kg.)	G.F.R. (ml/min)	Plasma pH	Plasma HCO_3 (mM/l)	Plasma pCO_2 (mmHg)	Urine flow (ml/min)	Urine pH	Urine HCO_3 (mM/l)	Urine pCO_2 (mmHg)	Bicarbonate Filt. (mM/min)	Excr. (mM/min)	Reabsrd. (mM/min)	Per 100 ml G.F. Excr. (mM)	Reabsrd. (mM)
1. 8.60	594	996	7.42	28.3	45	10.2	7.75	102.6	61	28.2	1.1	27.1	0.111	2.72
		946	7.42	28.7	46	9.0	7.76	124.7	71	27.2	1.1	27.1	0.116	2.76
7.9.60		918	7.40	26.5	44	15.4	7.59	58.3	58	24.3	0.9	23.4	0.098	2.55
		843	7.39	25.6	43	7.9	7.26	24.9	52	21.6	0.2	21.4	0.024	2.54
10.11.60		1111	7.42	26.9	43	11.3	7.71	115.5	71	29.9	1.3	28.6	0.098	2.57
		1256	7.42	26.9	43	16.3	7.89	176.7	74	33.8	2.9	36.9	0.229	2.46
Mean	594	1012	7.41	27.2	44	11.5	7.66	100.7	65	27.1	1.3	26.3	0.113	2.67
		±	±	±	±	±	±	±	±	±	±	±	±	±
S.D.		156	0.01	1.1	1	3.3	0.30	53.3	17	6.3	0.9	3.7	0.049	0.15

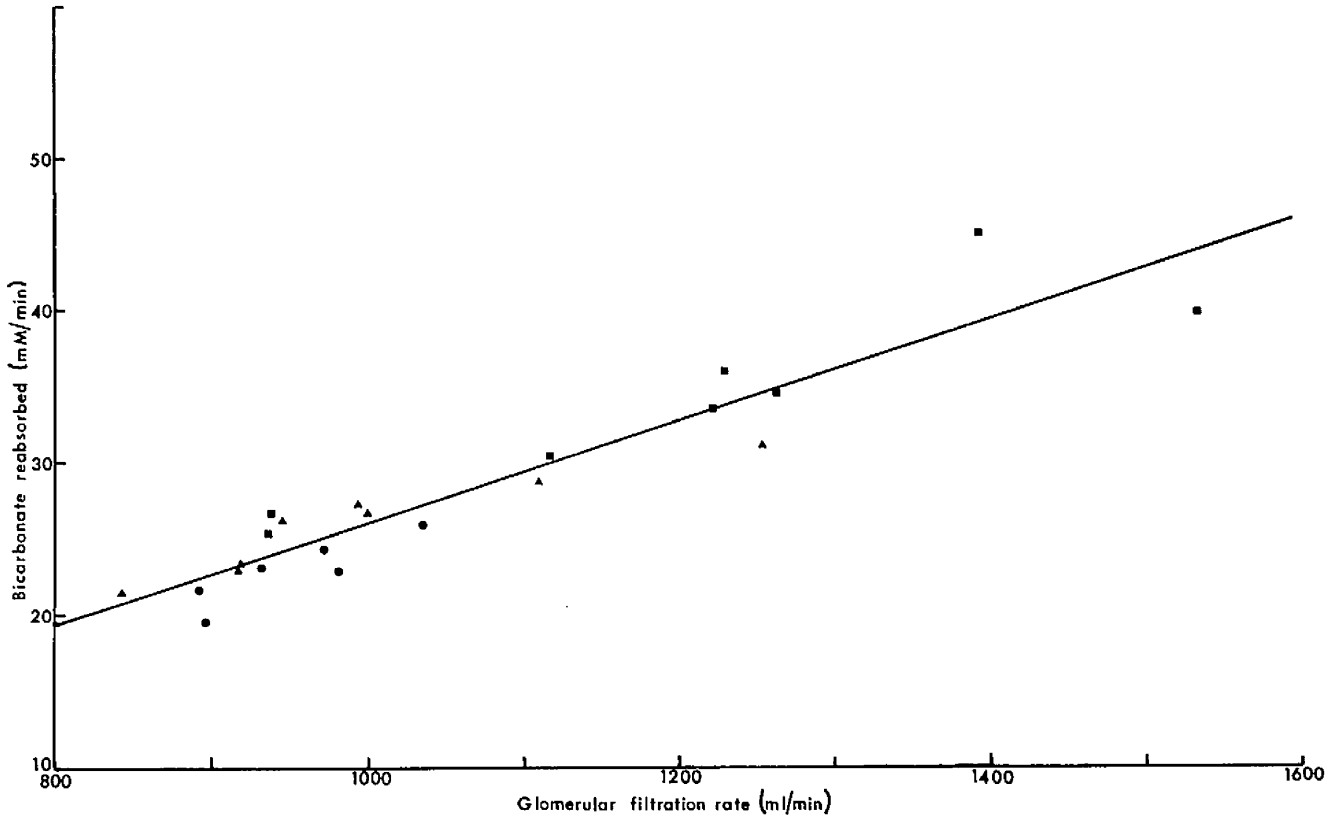


Fig. 26. The relationship of bicarbonate reabsorption rate (mM/min) to glomerular filtration rate.

three cows was 2.65 mM/100 ml. glomerular filtrate, and the mean rate of excretion was 0.087 mM/100 ml. of glomerular filtrate. It is therefore apparent that, despite the alkalinity of bovine urine, and the amount of bicarbonate which is at risk, only a very small proportion of the filtered load (3%) was actually excreted in these experiments.

In four experiments on Case no. 12916 (Table 33) bicarbonate excretion was exceptionally low despite normal or higher than normal plasma bicarbonate levels. Conversely, continued excretion of bicarbonate occurred when plasma levels were as low as 21.9 mM/l. (Case no. 14948, Table 34).

Fig. 27 shows bicarbonate excretion rate plotted against plasma concentration. While there is clearly no proportional relationship, it is apparent that many of the plasma concentrations in the clearance experiments on the cow fell within the normal range found in man. Despite this similarity of the normal plasma levels, the mean rate of bicarbonate excretion calculated from the present data was about 1.5 M/day in the cow, while that in man is normally about 2 mM/day. (Pitts & Lotspeich, 1946).

The mean urinary pCO_2 found in the urine samples from the three cows was 67 mm Hg. This shows close

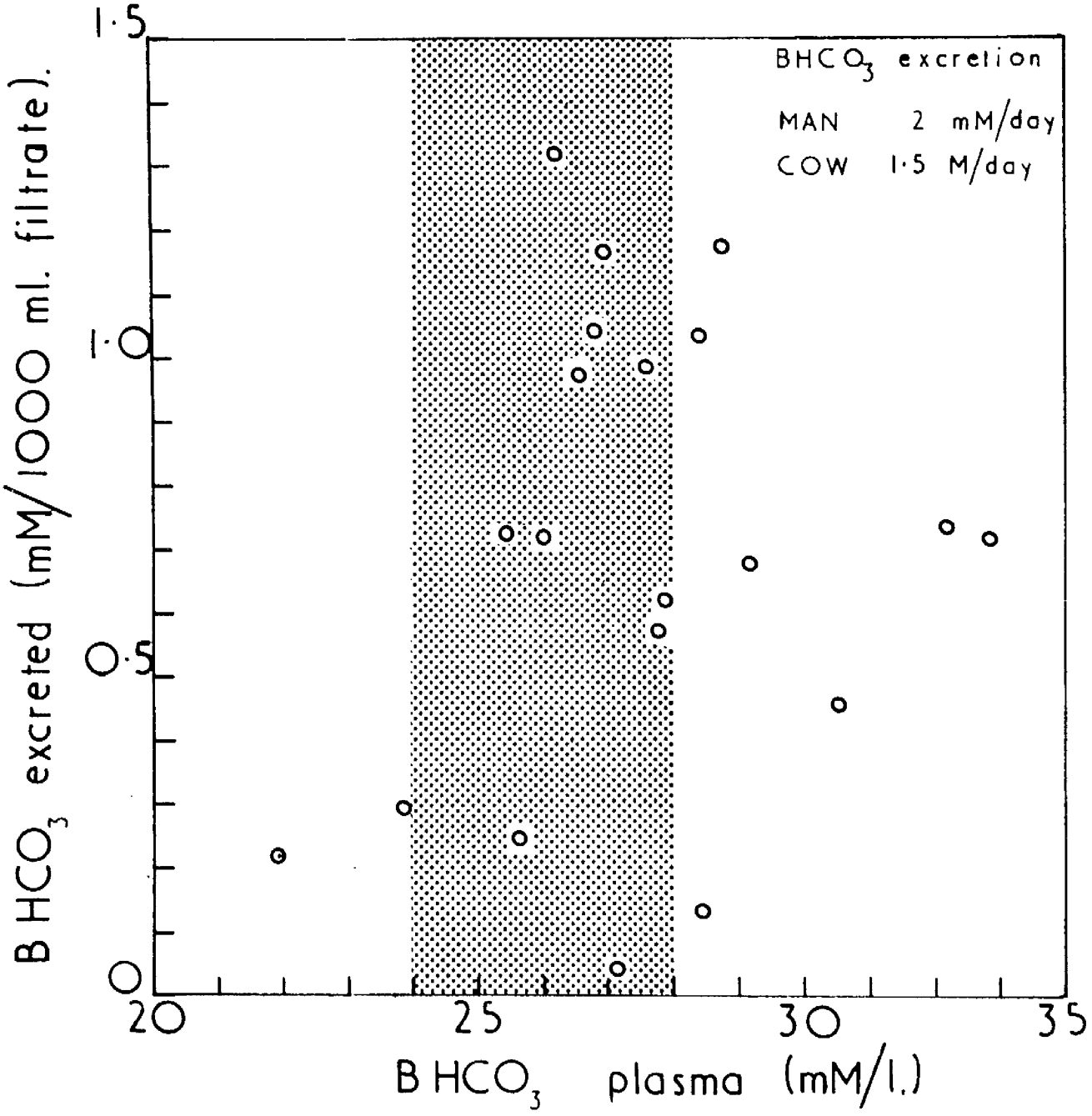


Fig. 27. The relationship of bicarbonate excretion rate to plasma bicarbonate concentration in the cow. The stippled band shows the normal range of plasma bicarbonate concentration in man.

agreement to the mean value of 64 mm Hg found in the survey of urine samples from a dairy herd. The pCO_2 values of arterial blood in all experiments remained fairly constant giving a mean value for the three cows of 44 mm Hg. In case no. 12916 (Table 33) the plasma pCO_2 was rather high in the first 4 measurements, but lattorly fell to a normal value. Plasma bicarbonate concentration and reabsorption rate was also high during the first two periods, suggesting the possibility of a compensated respiratory acidosis. There were, however, no other variations in plasma pCO_2 large enough to suggest that it played any part in the regulation of bicarbonate reabsorption in these experiments.

Section 4

The effect of acetazolamide and hydrochlorothiazide on the excretion of bicarbonate and other electrolytes

The effect of acetazolamide and hydrochlorothiazide on the excretion of bicarbonate and other electrolytes

The fundamental participation of carbonic anhydrase in the renal reabsorption of bicarbonate in man and the dog (Pitts, Ayer & Schiess, 1949; Brazeau & Gilman, 1953; Dorman, Sullivan & Pitts, 1954; Rector, Seldin, Roberts & Smith, 1960) suggested that further information on bicarbonate excretion in the cow might be obtained by administration of a carbonic anhydrase inhibitor during clearance measurements.

Measurements of glomerular filtration rate, and the filtration, reabsorption and excretion of sodium, potassium, bicarbonate and chloride were made before and after administration of the carbonic anhydrase inhibitor acetazolamide.* A further series of experiments on the effects of hydrochlorothiazide,† a widely used veterinary diuretic were compared and contrasted with the effects of acetazolamide. Although hydrochlorothiazide is often used in the treatment of oedematous conditions in cattle (Fluckiger & Hofer, 1960; Cowie, 1960; Newman, 1961), there is little

* Diamox. Cyanamid Co. of Great Britain.
† Votidrex. Ciba Laboratories Ltd.

critical information on its effect on electrolyte excretion in this species.

The work to be described was undertaken in collaboration with E.C. Pickering (Thesis, in preparation) and it was thus possible to obtain a more composite picture of renal electrolyte excretion than has hitherto been described.

METHOD

Clearance technique was as described previously with the following exceptions. The measurement of pH in urine samples was made in a capillary glass electrode system enclosed in a water jacket through which water was pumped at a controlled temperature of 39°C (E.I.L. Replaceable Capillary Glass Electrode System Model SHH 33). This system replaced the electrode system used previously (E.I.L. - Mondel Electrode System). During these experiments, pH measurement of whole blood was not sufficiently accurate to be recorded, thus pH and bicarbonate values in arterial blood were not obtained. Urine pH values were more reliable and total CO_2 measurements were carried out on the manometric apparatus as described previously. Chloride measurements were made on a chloride meter* and sodium

* E.E.L.

and potassium estimations made on a flame photometer*.

After a control period, acetazolamide was administered intravenously at a dosage of 5 mg/Kg (approximately 2 g per cow) and hydrochlorothiazide was injected intramuscularly according to the manufacturers recommendation at a dose rate of 250 mg per cow.

RESULTS

The results of 12 experiments (8 on acetazolamide, and 4 on hydrochlorothiazide) on three cows are recorded.

Urine flow In all experiments there was a marked and rapid diuretic response to the administration of either drug. The effect of administration of acetazolamide and hydrochlorothiazide on the urine flow, and the total concentration of the principal urinary electrolytes (Na^+, K^+, $Cl.^-$, HCO_3^-) is shown on Figs. 28 & 29. Similar responses to these occurred in all other experiments. The increased rate of flow resulting from drug administration occurred within 10 min of the injection of either drug, thus the intramuscular route of administration of hydrochlorothiazide did not

* E.E.L.

Fig. 28. The effect of acetazolamide and hydrochlorothiazide on urine flow and total concentration of urinary electrolytes. Case no. 21270 (355 Kg). 2.0 g acetazolamide or 250 mg hydrochlorothiazide were given at the arrows. In the lower left figure, an additional 4.0 g acetazolamide was infused during the 60 min after the initial dose. The horizontal broken lines are drawn through the mean pre-dosing values of urine flow with the increase in urine volume noted above the line, and the total increase noted separately.

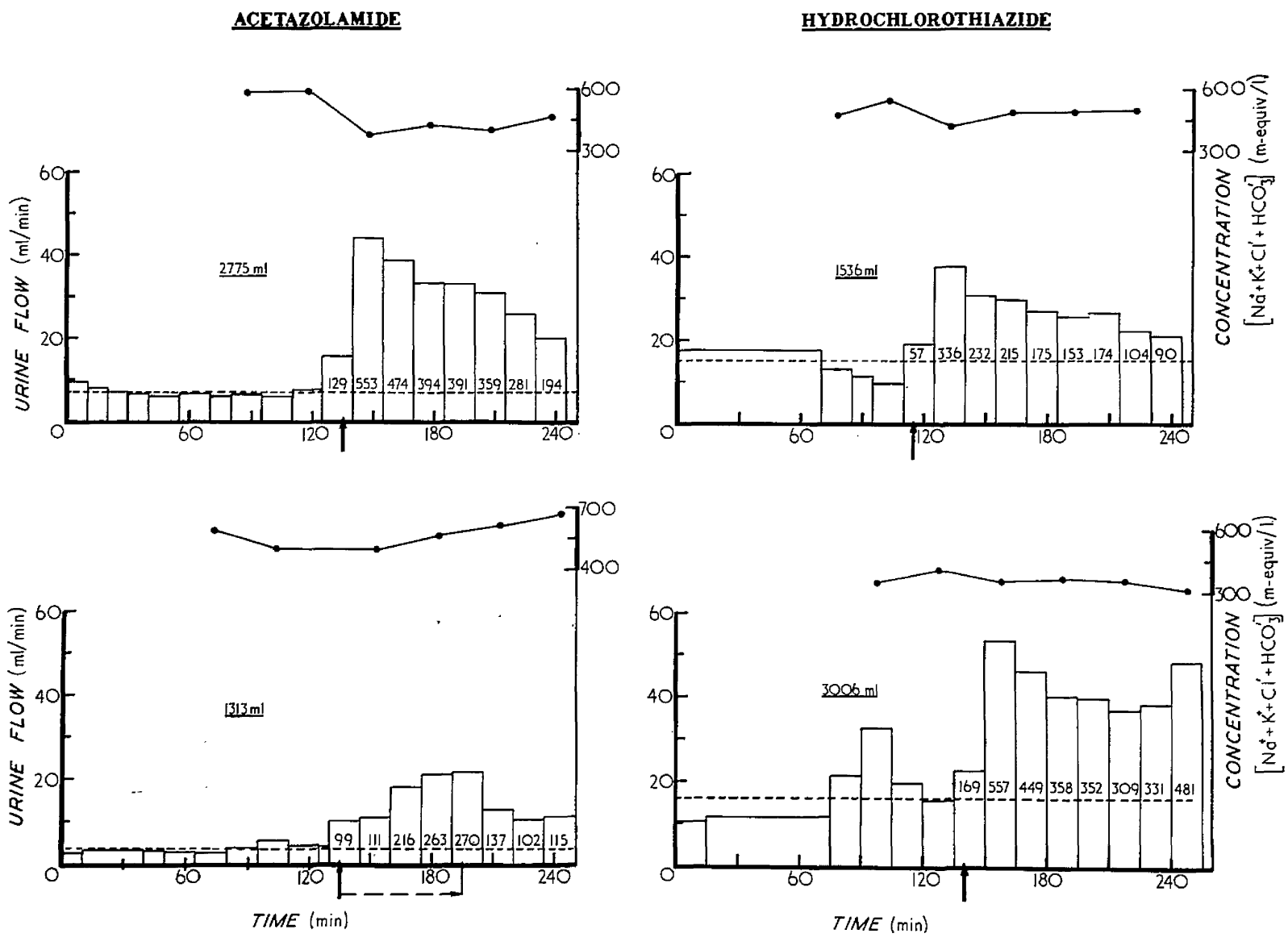
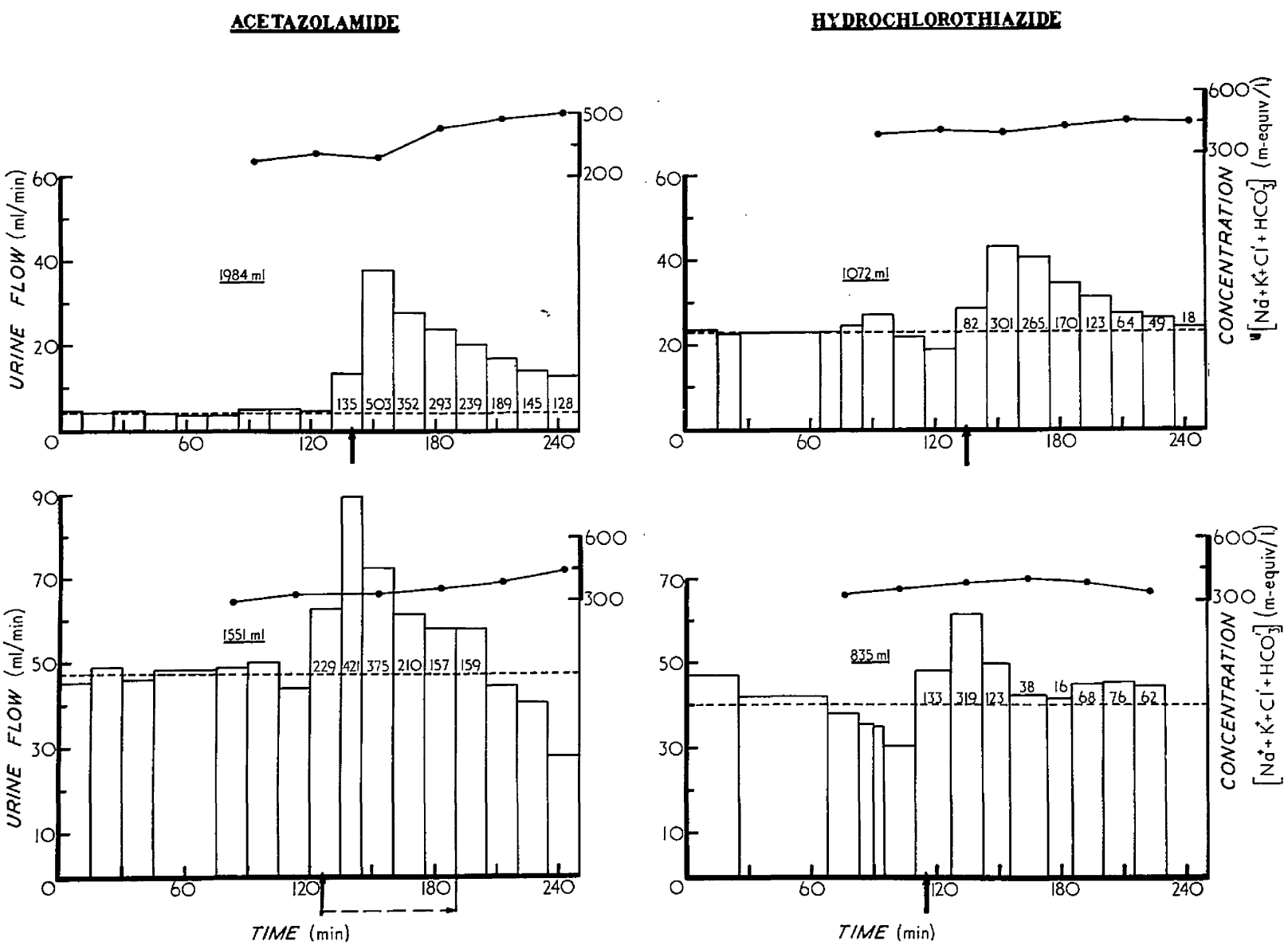

ACETAZOLAMIDE
HYDROCHLOROTHIAZIDE
URINE FLOW (ml/min)
CONCENTRATION [Na+K+Cl+HCO3] (m-equiv/l)
2775 ml
129 553 474 394 391 359 281 194
1536 ml
57 336 232 215 175 153 174 104 90
1313 ml
99 111 216 263 270 137 102 115
3006 ml
169 557 449 358 352 309 331 481
TIME (min)

Fig. 29. The effect of acetazolamide on urine flow and the total concentration of urinary electrolytes. Case no. 21271 (376 Kg). 2.0 g acetazolamide or 250 mg hydrochlorothiazide were given at the arrows. In the lower left figure, an additional 4.0 g acetazolamide was infused during the 60 min after the initial dose. The horizontal broken lines are drawn through the mean pre-dosing values of urine flow, with the increase in urine volume noted above the line, and the total increase noted separately.


ACETAZOLAMIDE
HYDROCHLOROTHIAZIDE
URINE FLOW (ml/min)
CONCENTRATION [Na+K+Cl+HCO3] (m-equiv/l)
1984 ml
135 503 352 293 239 189 145 128
1072 ml
82 301 265 170 123 64 49 18
1551 ml
229 421 375 210 157 159
835 ml
133 319 123 38 16 68 76 62
TIME (min)

cause a slower response than the intravenous administration of acetazolamide. The time of the attainment of peak diuresis was variable, but, in most experiments, occurred within 20 min of drug administration. The diuretic response to each drug was sustained for a variable length of time. Where the initial rate of urine flow was low, the response was usually sustained longer than when the initial rate of flow was high. Infusion of acetazolamide after the priming injection did not sustain the increase in the rate of urine flow, (Fig. 29) and in this experiment, flow rate fell below the predosing rate soon after stopping infusion of the drug.

In each figure, the mean flow rate before the drug is shown by the interrupted line. An approximate estimation of the volume of fluid excreted as a result of the drug administration is given by the volumes above this line on the histogram. The total volume excreted in excess of the mean predosing flow rate is shown underlined in each figure. The mean volume excreted due to acetazolamide was 1986 (853 - 3504)ml. and to hydrochlorothiazide was 1812 (853 - 3006)ml. Thus both drugs had a similar quantitative effect on urine flow at the dose rates used. There was no apparent relationship between the predosing flow rate

and the volume of excretion caused by the drug, but there was a relationship between the pre-dosing flow rate and the percentage diuresis which occurred (Fig.30). An inverse asymptotic relationship is shown between pre-dosing flow-rate, and percentage diuresis. Figs. 29 and 30 also show the relationship of the total concentration of the main urinary electrolytes (Na^+, K^+, $Cl.^-$, HCO_3^-) to the increased flow rate. It is apparent that there is no dilution of the electrolyte concentration as a result of the increased urine volume and therefore the diuretic responses do not represent a loss of osmotically free water, but rather an increased salt excretion accompanied secondarily by increased water loss.

Electrolyte excretion The effect of each drug on electrolyte excretion and urine pH is shown on Figs. 31 and 32. The results shown were all from the same animal kept under uniform conditions of management. Acetazolamide caused a sharp increase in excretion of sodium and bicarbonate, and less marked increase in potassium and chloride (Fig. 31(a) and (b), whereas after hydrochlorothiazide there was a marked increase in chloride excretion accompanying the natriuresis, and a smaller increase in potassium, and bicarbonate excretion. The same effect is shown in Fig. 32.

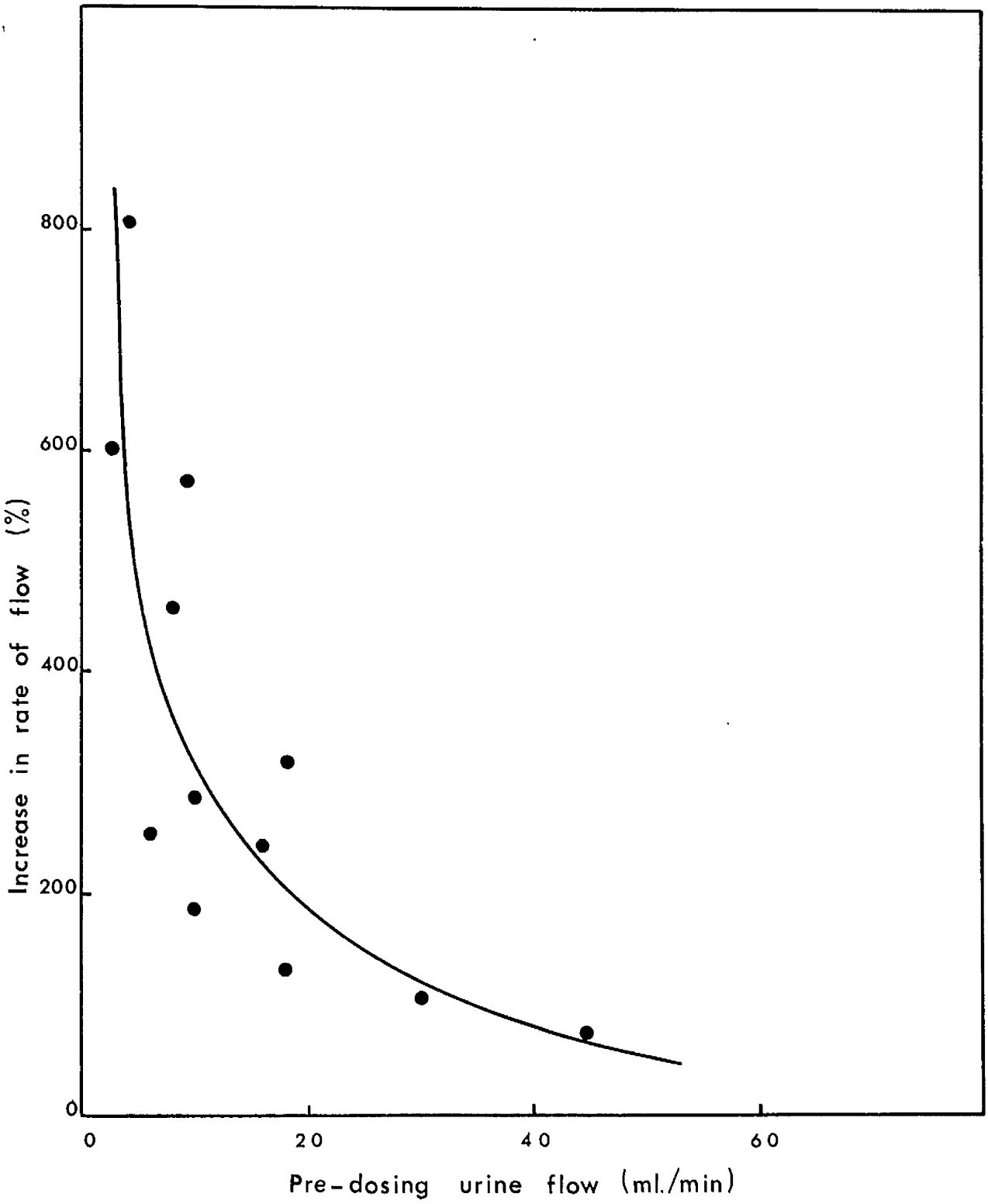


Fig. 30. Relationship of the pre-dosing rate of urine flow to the percentage diuresis in response to administration of acetazolamide or hydrochlorothiazide.

Fig. 31. The effect of acetazolamide and hydrochorothiazide on the rate of excretion of urinary electrolytes and on urinary pH. Case no. 21270 (355 Kg.), 2.0 g acetazolamide or 250 mg hydrochlorothiazide were given at the arrows. In the lower left figure, an additional 4.0 g acetazolamide was infused during the 60 min after the initial dose. Values are plotted at the mid-point of 15 mins clearance periods.

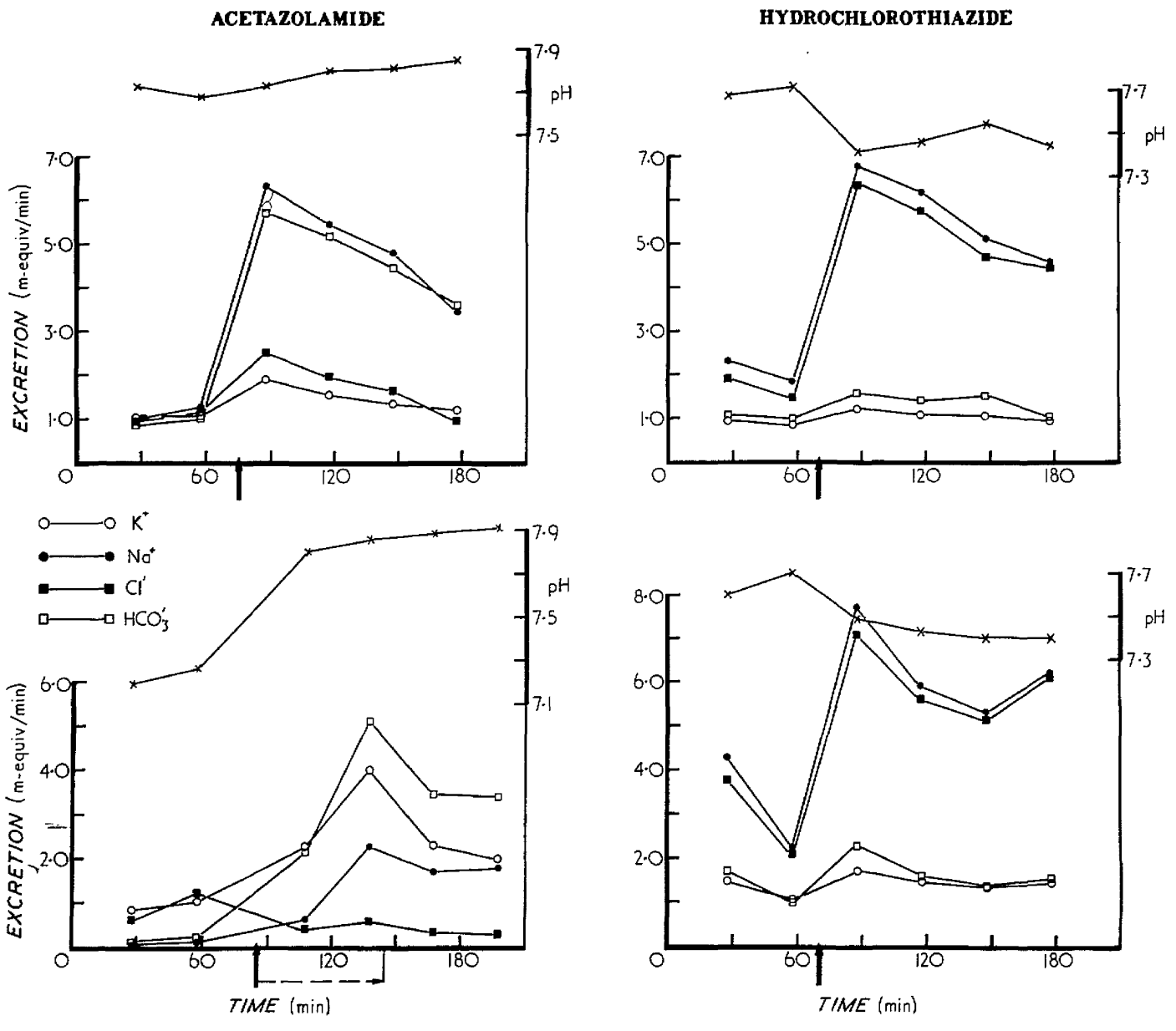
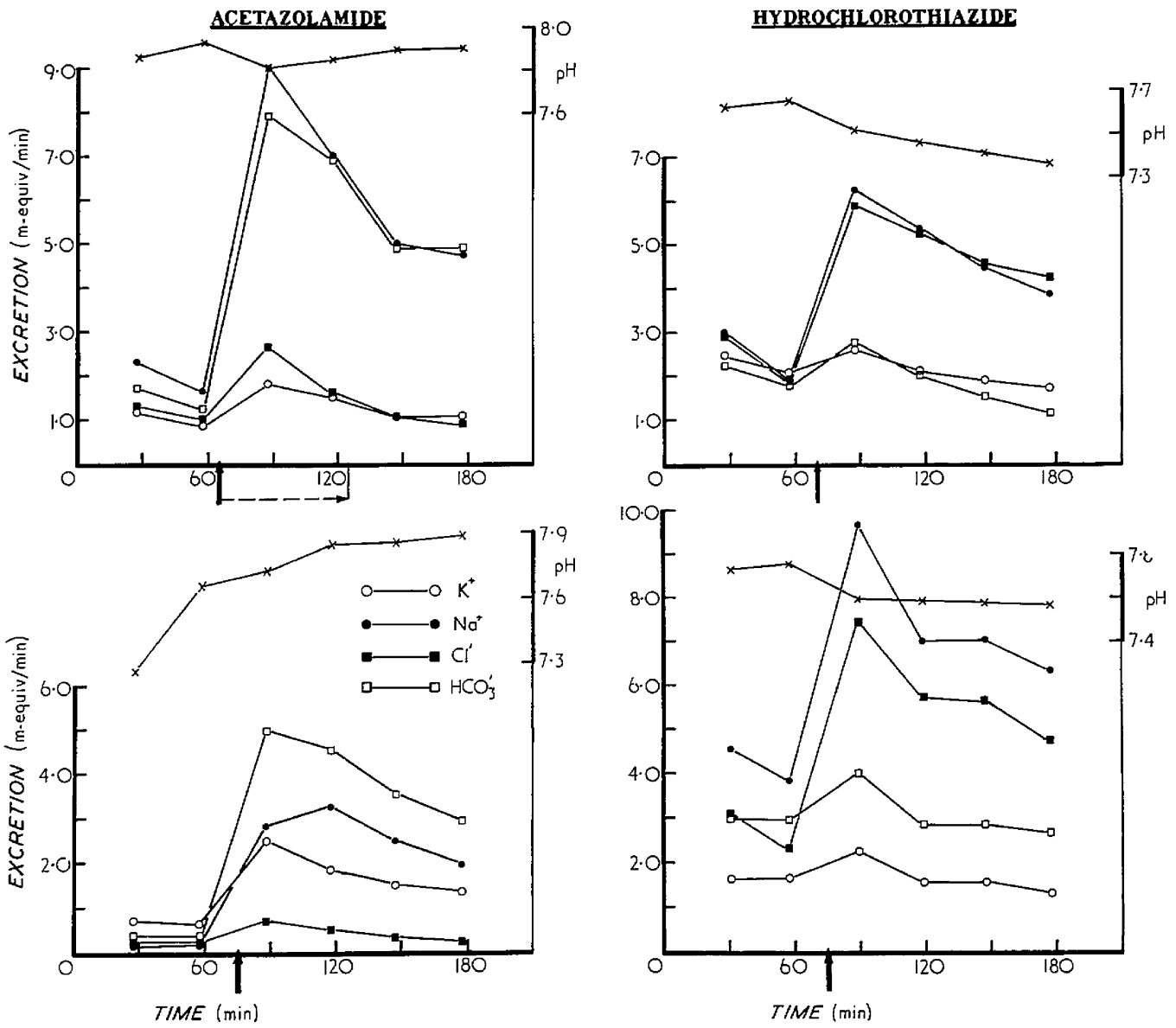

ACETAZOLAMIDE
HYDROCHLOROTHIAZIDE
EXCRETION (m-equiv/min)
pH
TIME (min)
K+
Na+
Cl'
HCO3'
7·9
7·5
7·1
7·7
7·3
7·0
5·0
3·0
1·0
6·0
4·0
2·0
8·0
0
60
120
180

Fig. 32. The effect of acetazolamide and hydrochorothiazide on the rate of excretion of urinary electrolytes and on urinary pH. Case no. 21271 (376 Kg). 2.0 g acetazolamide or 250 mg hydrochorothiazide were given at the arrows. In the upper left figure an additional 4.0 g acetazolamide was infused during the 60 min after the initial dose. Values are plotted at the mid-point of 15 min clearance periods.


ACETAZOLAMIDE
HYDROCHLOROTHIAZIDE
EXCRETION (m-equiv/min)
pH
K+
Na+
Cl'
HCO3'
TIME (min)
0
60
120
180

With the exception of Fig 31 (a), the increase in bicarbonate excretion was accompanied by a marked increase in urine pH. In this figure the increased bicarbonate was largely attributable to increased rate of urine flow rather than increased bicarbonate concentration, and thus there was little variation in urine pH. After hydrochlorthiazide, the increased rate of urine flow was invariably accompanied by a fall in urine pH.

CO_2 tension After administration of acetazolamide, the increased bicarbonate excretion was accompanied by an increase in the urinary CO_2 tension. Urinary pCO_2 values from two representative experiments are shown on Fig 33. There was no change in urinary pCO_2 values after hydrochlorothiazide.

Glomerular filtration rate There is an apparent marked fall in glomerular filtration rate as a result of acetazolamide administration, while after hydrochlorothiazide, filtration rate was unaltered. Fig. 34 shows the results of filtration rate measurements in four experiments.

Plasma electrolytes Little variation in plasma electrolytes was seen after either drug, with the exception that there was a slight, but consistent fall in plasma potassium concentration after administration

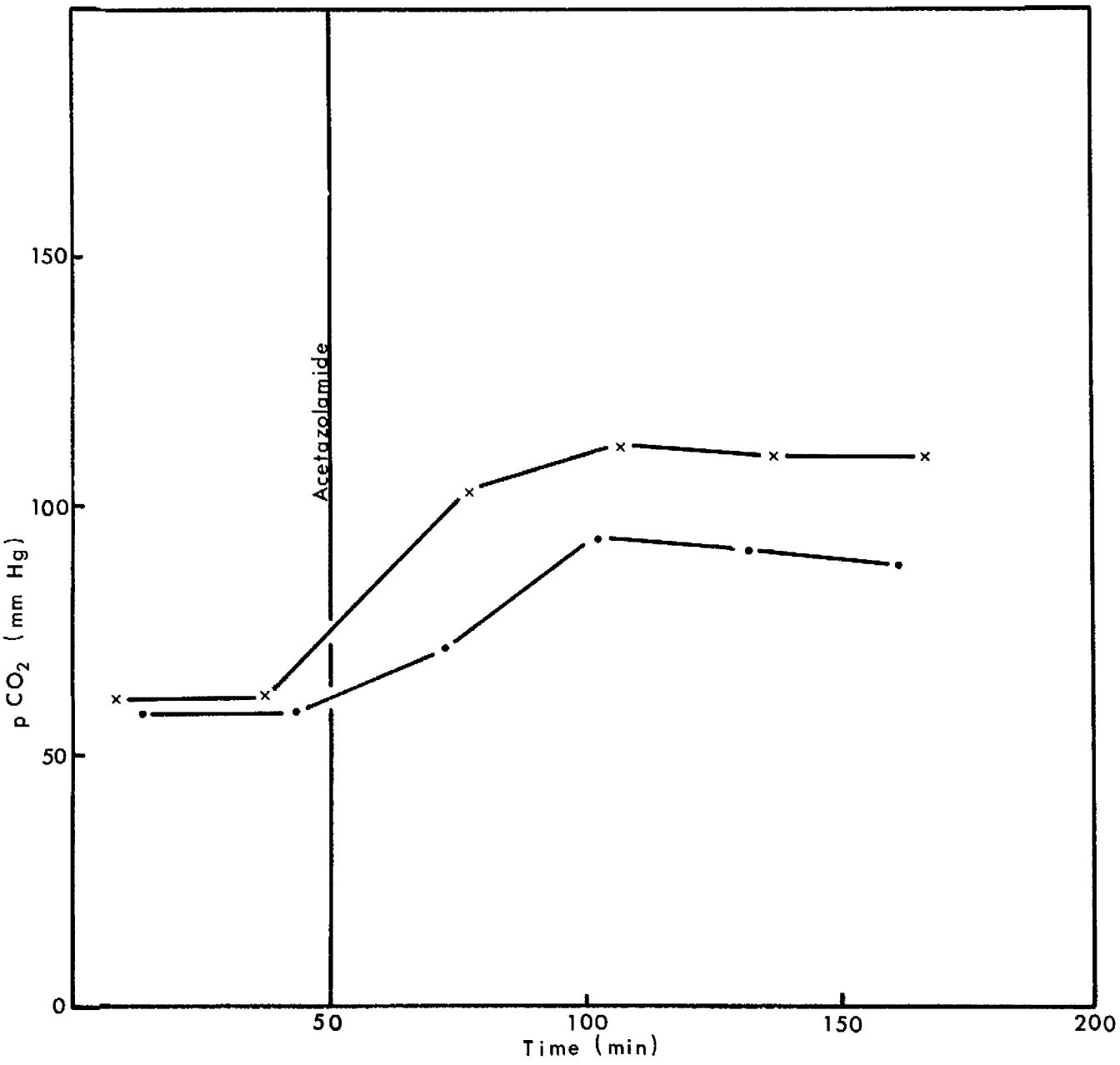


Fig. 33. Effect of acetazolamide on urinary pCO_2 values in two representative experiments. 2.0g of acetazolamide was given at 50 min.

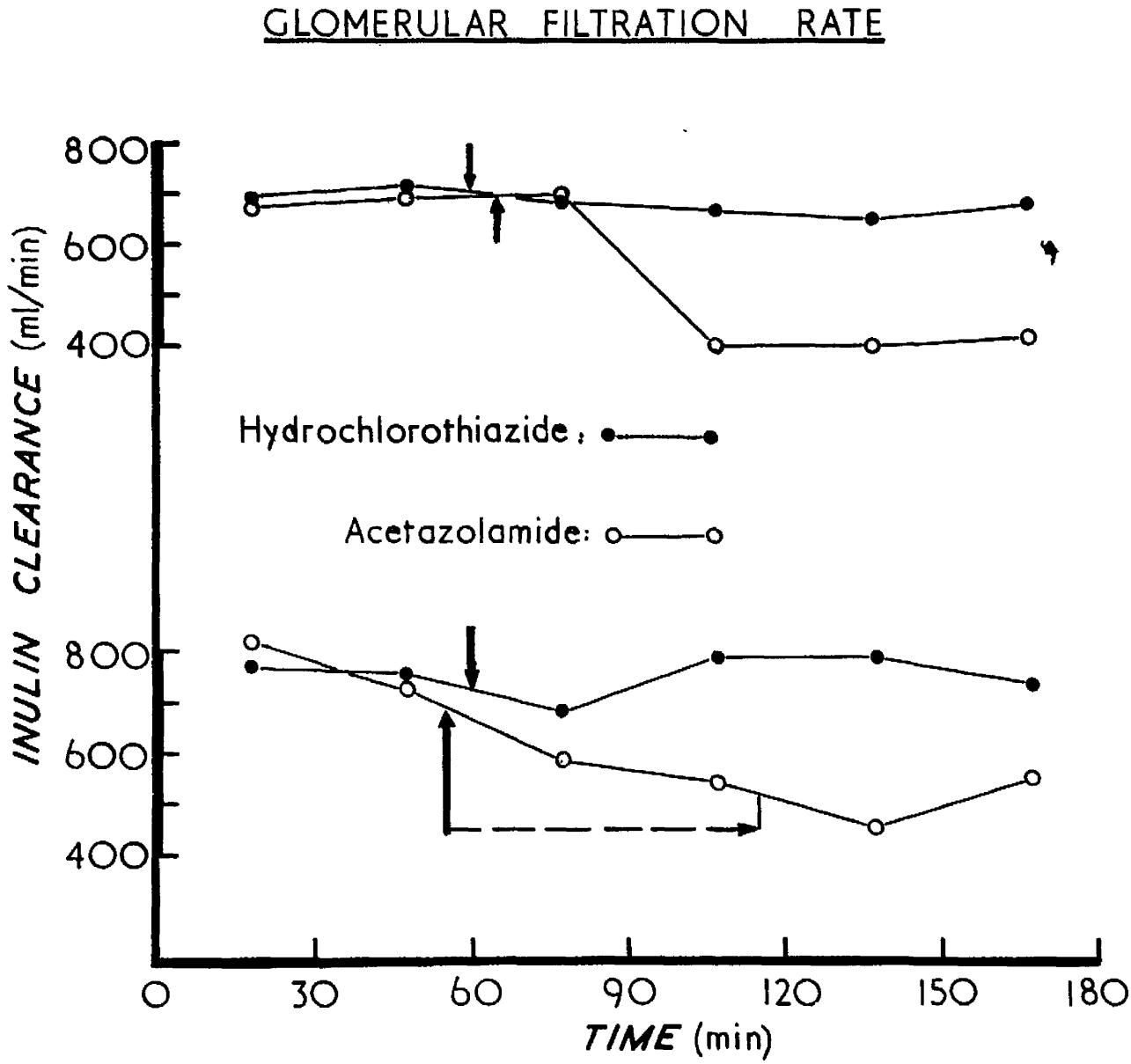


Fig. 34. The effect of acetazolamide and hydrochlorothiazide on glomerular filtration rate. Values are plotted at the mid-point of 15 min clearance periods for case no. 21270 (355 Kg) in the upper figures, and for case no. 21271 (376 Kg) in the lower figures. 2.0 g acetazolamide or 250 mg hydrochlorothiazide were given at the arrows, with an additional 4.0 g acetazolamide infused during the 60 min after the initial dose in case no. 21271.

of hydrochlorothiazide.

Collected results The collected results of all these experiments are shown in Tables 36 to 47.

Table 36

Case number 21271 Weight 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 HCO_3^- (mM/l)	Urine Cl^- (mM/l)	Urine pH	Urine pCO_2 (mmHg)	Urine Vol (ml/min)	G.F.R. (ml/min)	Excretion Na^+ (mM/min)	Excretion K^+ (mM/min)	Excretion HCO_3^- (mM/min)	Excretion Cl^- (mM/min)
-45 to -30	141.3	2.7	96.0	167.5	85.0	124.8	96.5	7.85	58	13.73	818	2.300	1.167	1.714	1.325
-15 to 0	141.3	2.7	97.0	180.0	94.0	136.1	111.0	7.92	58	9.20	726	1.656	0.865	1.252	1.021
0	2g acetazolamide i/v (loading dose) Infuse acetazolamide at 66 mg/min														
15-30	141.3	2.5	97.5	151.3	30.0	132.7	44.5	7.80	71	59.60	582	9.018	1.788	7.909	2.652
45-60	141.3	2.4	98.0	170.0	36.5	167.8	39.5	7.84	94	41.27	546	7.016	1.506	6.925	1.630
60	Stop infusion														
75-90	141.3	2.4	100.5	175.0	37.0	173.2	37.0	7.89	91	28.53	457	4.993	1.056	4.941	1.053
105-120	141.3	2.5	101.5	186.3	43.0	193.9	35.0	7.90	87	25.47	553	4.745	1.095	4.939	0.891

Table 37

Case number 21270 Weight 382 Kg.

Time (min)	Plasma Na^+ (mM/l)	Plasma K^+ (mM/l)	Plasma Cl^- (mM/l)	Urine Na^+ (mM/l)	Urine K^+ (mM/l)	Urine HCO_3^- (mM/l)	Urine Cl^- (mM/l)	pH	pCO_2 (mmHg)	Vol. (ml/min)	G.F.R. (ml/min)	Excretion Na^+ (mM/min)	Excretion K^+ (mM/min)	Excretion HCO_3^- (mM/min)	Excretion Cl^- (mM/min)
-65 to -50	146.3	3.9		15.5	313.6	41.0	221.0	7.19	94	2.67	532	0.041	0.838	0.109	0.590
-35 to -20	146.3	3.9	112.0	17.0	237.5	36.8	213.5	7.26	81	5.60	697	0.095	1.330	0.206	1.196
0			2g acetazolamide i/v (loading dose)					Infuse acetazolamide at 100 mg/min							
15-30	146.3	3.2	110.5	58.0	207.5	197.7	34.5	7.80	123	10.93	370	0.634	2.268	2.160	0.377
45-60	146.3	3.1	111.0	107.5	190.0	243.2	25.5	7.86	126	21.07	563	2.265	4.003	5.124	0.537
60								Stop infusion							
75-90	146.3	3.1	111.5	133.8	180.0	272.0	23.5	7.88	129	12.67	419	1.695	2.281	3.446	0.298
105-120	146.3	3.1	110.0	158.8	181.3	302.9	23.5	7.91	133	11.20	428	1.778	2.030	3.392	0.263

Table 38

Case number 21271 Weight 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 HCO_3^- (mM/l)	Urine Cl^- (mM/l)	pH	pCO_2 (mmHg)	Vol. (ml/min)	G.F.R. (ml/min)	Excretion Na^+ (mM/min)	Excretion K^+ (mM/min)	Excretion HCO_3^- (mM/min)	Excretion Cl^- (mM/min)
-50 to -35	135.0	2.4	96.5	92.5	56.5	56.0	86.5	7.54	71	40.13	495	3.712	2.267	2.246	3.471
-20 to -4	138.8	2.3	97.5	108.8	56.8	52.2	107.5	7.50	71	43.93	333	4.780	2.495	2.292	4.723
0	2g acetazolamide i/v (loading dose) Infuse acetazolamide at 66 mg/min														
20-35	135.0	2.1	96.0	132.5	33.0	107.6	55.5	7.66	104	72.53	439	9.610	2.393	7.793	4.025
50-65	132.5	2.0	98.5	135.0	38.0	126.0	53.5	7.68	113	58.00	286	7.830	2.204	7.308	3.103
65	Stop infusion														
80-95	130.0	1.9	98.0	146.3	43.5	147.6	52.5	7.75	110	44.47	534	6.506	1.934	6.568	2.335
110-125	130.0	1.9	98.0	155.0	65.6	176.9	45.5	7.82	110	28.00	334	4.340	1.834	4.953	1.274

Table 39

Case number 21271 Weight 355 Kg.

Time (min)	Plasma			Urine								Excretion			
	Na^+ (mM/l)	K^+ (mM/l)	Cl^- (mM/l)	Na^+ (mM/l)	K^+ (mM/l)	HCO_3 (mM/l)	Cl^- (mM/l)	pH	pCO_2 (mmHg)	Vol. (ml/min)	G.F.R. (ml/min)	Na^+ (mM/min)	K^+ (mM/min)	HCO_3 (mM/min)	Cl^- (mM/min
-55 to -40	142.5	2.4	103.0	102.0	75.0	62.2	113.0	7.58	71	29.5	1030	3.012	2.215	1.837	3.337
-25 to -10	142.5	2.7	109.0	112.5	92.0	83.9	123.5	7.63	81	19.3	897	2.171	1.776	1.619	2.384
0	2.0 g of acetazolamide in 20 ml of water by rapid i/v injection														
-20	143.8	2.4	107.5	125.0	39.0	120.1	49.5	7.67	129	78.7	657	9.841	3.070	9.455	3.897
5-50	141.3	2.6	109.5	146.0	48.0	142.6	55.5	7.74	104	56.1	788	8.186	2.691	7.996	3.112
5-80	138.8	3.0	109.5	157.5	62.5	157.1	57.5	7.74	113	40.1	777	6.311	2.504	6.295	2.304
5-110	138.8	2.5	108.0	161.3	86.0	200.1	49.0	7.80	123	24.4	678	3.936	2.098	4.882	1.196

Table 40

Case number 21271 Weight 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 HCO_3^- (mM/l)	Urine Cl^- (mM/l)	Urine pH	Urine pCO_2 (mmHg)	Urine Vol. (ml/min)	G.F.R. (ml/min)	Excretion Na^+ (mM/min)	Excretion K^+ (mM/min)	Excretion HCO_3^- (mM/min)	Excretion Cl^- (mM/min)
5 to 0	138.8	3.3	100.5	19.5	145.0	68.4	39.5	7.57	81	4.87	932	0.095	0.706	0.333	0.192
5 to 0	138.8	3.3	103.5	36.5	141.3	67.8	46.5	7.65	84	4.40	728	0.161	0.623	0.386	0.205
			2.0 g acetazolamide in 20 ml water by rapid i/v injection												
20	140.0	3.0	102.5	74.0	65.5	130.9	18.0	7.72	113	37.73	566	2.792	2.471	4.939	0.679
-50	140.0	2.8	103.5	137.5	77.0	191.0	21.5	7.84	116	23.73	515	3.263	1.827	4.532	0.510
-80	138.8	2.9	103.0	147.5	89.0	209.2	19.5	7.85	116	16.80	492	2.478	1.495	3.515	0.238
-110	138.8	2.8	107.5	148.8	105.0	227.9	17.0	7.88	116	12.73	610	1.894	1.337	2.901	0.216

Table 41

Case number 21270 — Weight 376 Kg.

Time (min)	Plasma Na^+ (mM/l)	Plasma K^+ (mM/l)	Plasma Cl^- (mM/l)	Urine Na^+ (mM/l)	Urine K^+ (mM/l)	Urine HCO_3^- (mM/l)	Urine Cl^- (mM/l)	Urine pH	Urine pCO_2 (mmHg)	Urine Vol. (ml/min)	Urine G.F.R. (ml/min)	Excretion Na^+ (mM/min)	Excretion K^+ (mM/min)	Excretion HCO_3^- (mM/min)	Excretion Cl^- (mM/min)
-55 to -40	143.8	3.0	101.5	153.8	155.0	131.8	142.5	7.72	91	6.60	670	1.015	1.023	0.870	0.941
-25 to -10	143.8	3.3	104.5	165.0	141.3	129.7	152.5	7.67	100	7.67	689	1.266	1.084	0.9950	1.170
0	2.25 g acetazolamide in 20 ml water by rapid i/v injection														
5-20	145.0	3.0	105.0	143.8	42.8	130.0	56.5	7.72	104	43.80	697	6.298	1.875	5.694	2.475
35-50	145.0	3.2	106.5	163.8	46.0	155.5	58.5	7.79	104	33.23	399	5.438	1.527	5.163	1.942
65-80	143.8	2.8	105.5	155.0	43.0	144.1	52.5	7.81	94	30.87	401	4.785	1.327	4.448	1.621
95-110	143.8	2.8	103.5	173.8	59.5	182.5	47.5	7.84	104	19.67	414	3.453	1.182	3.626	0.944

Table 42

Case number 20636 Weight 412 Kg.

Time (min)	Plasma Na^+ (mM/l)	Plasma K^+ (mM/l)	Plasma Cl^- (mM/l)	Urine Na^+ (mM/l)	Urine K^+ (mM/l)	Urine HCO_3^- (mM/l)	Urine Cl^- (mM/l)	pH	pCO_2 (mmHG)	Vol. (ml/min)	G.F.R. (ml/min)	Excretion Na^+ (mM/min)	Excretion K^+ (mM/min)	Excretion HCO_3^- (mM/min)	Excretion Cl^- (mM/min)
49 to 33	143.7	2.5	98.0	59.5	139.0	92.3	58.5	7.57	65	9.88	909	0.588	1.373	0.912	0.578
	2 g acetazolamide i/v (loading dose) Infuse acetazolamide at 27 mg/min														
5-45	143.7	2.4	99.0	173.5	87.0	117.1	11.0	7.78	74	28.35	578	4.919	2.466	3.320	0.312
5-115	143.7	2.2	100.0	187.3	117.5	275.1	8.5	7.79	162	17.80	643	3.338	2.092	4.897	0.151
60-175	143.7	2.3	101.0	200.0	152.5	307.2	7.5	7.83	162	13.13	840	2.626	2.002	4.034	0.098

Table 43

Case number 21270 Weight 376 Kg.

Time (min)	Plasma Na^+ (mM/l)	Plasma K^+ (mM/l)	Plasma Cl^- (mM/l)	Urine Na^+ (mM/l)	Urine K^+ (mM/l)	Urine HCO_3^- (mM/l)	Urine Cl^- (mM/l)	pH	pCO_2 (mmHg)	Vol. (ml/min)	G.F.R. (ml/min)	Excretion Na^+ (mM/min)	Excretion K^+ (mM/min)	Excretion HCO_3 (mM/min)	Excretion Cl^- (mM/min)
30 to 55	145.0	3.1		197.5	207.5		233.0		4.40	670		0.869	0.913		1.025
50 to 85	145.0	3.1		161.3	187.5		189.0		4.87	599		0.786	0.913		0.926
80 to 5	143.8	3.1		162.5	235.0		219.0		2.73	507		0.444	0.642		0.591
)	2 g acetazolamide in 20 ml water by rapid i/v injection														
)-25	145.0	3.1		170.0	87.5		34.0		21.13	527		3.592	1.849		0.717
)-55	143.8	2.9		210.3	105.0		33.5		21.67	711		4.551	2.275		0.724
)-85	143.8	3.0		230.0	117.5		26.0		7.67	310		1.764	0.901		0.200

Table 44

Case number 21271 — Weight 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 HCO_3 (mM/l)	Urine Cl^- (mM/l)	Urine pH	Urine pCO_2 (mmHg)	Urine Vol. (ml/min)	G.F.R. (ml/min)	Excretion Na^+ (mM/min)	Excretion K^+ (mM/min)	Excretion HCO_3 (mM/min)	Excretion Cl^- (mM/min)
0 to 5	140.0	2.4	98.5	111.0	90.5	81.9	108.5	7.62	81	27.13	770	3.011	2.455	2.222	2.944
0 to 0	138.8	2.4	97.5	103.0	111.5	95.2	103.0	7.65	87	18.73	775	1.929	2.088	1.783	1.929
				5 ml of hydrochlorthizide (250 mg) intramuscularly.											
-25	138.8	2.4	95.5	145.0	60.0	63.8	136.0	7.52	81	43.00	677	6.235	2.580	2.743	5.848
-55	138.8	2.4	98.5	156.0	61.0	59.2	153.5	7.50	84	34.27	788	5.346	2.091	2.029	5.260
-85	140.0	2.4	95.5	165.0	69.5	55.4	166.5	7.41	84	27.20	787	4.488	1.890	1.507	4.529
0-115	138.8	2.5	89.5	160.0	70.5	47.7	175.5	7.36	81	24.13	732	3.861	1.701	1.151	4.235

Table 45

Case number 21270 Weight 376 Kg.

Time (min)	Plasma			Urine							C.F.R.	Excretion			
	Na^+ (mM/l)	K^+ (mM/l)	Cl^- (mM/l)	Na^+ (mM/l)	K^+ (mM/l)	HCO_3 (mM/l)	Cl^- (mM/l)	pH	pCO_2 (mm Hg)	Vol. (ml/min)	(ml/min)	Na^+ (mM/min)	K^+ (mM/min)	HCO_3 (mM/min)	Cl^- (mM/min)
-50 to -35	141.3	2.7	103.5	132.3	45.5	52.9	115.5	7.60	55	32.40	347	4.267	1.474	1.714	3.742
-20 to -5	141.3	2.7	101.5	143.0	67.0	66.3	133.5	7.70	55	15.60	245	2.231	1.045	1.034	2.083
0	5 ml hydrochlorthiazide (250 mg) intramuscularly														
10-25	141.3	2.5	101.0	144.5	32.0	43.4	133.0	7.49	58	53.33	301	7.706	1.707	2.315	7.093
40-55	141.3	2.8	103.0	147.0	35.8	40.1	139.5	7.43	65	40.07	302	5.890	1.475	1.607	5.590
70-85	141.3	2.5	98.5	143.8	36.3	37.6	139.0	7.40	65	36.80	283	5.292	1.336	1.384	5.115
100-115	141.3	2.7	99.0	128.5	30.0	32.0	124.5	7.40	55	48.27	498	6.203	1.448	1.545	6.010

Table 46

Case number 21271 Weight 355 Kg.

Time (min)	Plasma Na^+ (mEq/l)	Plasma K^+ (mEq/l)	Plasma Cl^- (mEq/l)	Urine Na^+ (mEq/l)	Urine K^+ (mEq/l)	Urine HCO_3^- (mEq/l)	Urine Cl^- (mEq/l)	pH	pCO_2 (mmHg)	Vol. (ml/min)	C.P.R. (ml/min)	Excretion Na^+ (mEq/min)	Excretion K^+ (mEq/min)	Excretion HCO_3^- (mEq/min)	Excretion Cl^- (mEq/min)
-47 to -32	139.0	2.4	89.5	120.0	43.0	79.3	80.5	7.73	65	38.00	600	4.560	1.634	3.013	3.059
-20 to -5	139.0	2.3	93.5	126.0	54.5	96.5	76.5	7.75	74	30.47	598	3.839	1.661	2.940	2.331
0	5 ml of hydrochlorthiazide (250 mg) intremuscularly.														
12-27	139.0	2.3	89.5	157.0	36.5	64.9	120.5	7.59	68	61.53	613	9.660	2.246	3.993	7.414
40-55	139.0	2.3	88.0	165.0	37.0	67.3	135.0	7.58	71	42.33	594	6.985	1.566	2.849	5.715
70-85	139.0	2.4	90.5	157.5	35.0	64.1	125.5	7.58	71	51.80	695	7.656	1.568	2.872	5.622
100-115	139.0	2.4	90.5	142.5	38.0	68.5	106.5	7.56	71	44.40	686	6.327	1.332	2.686	4.729

Table 47

Case number 21270 Weight 376 Kg.

Time (min)	Plasma Na^+ (mM/l)	Plasma K^+ (mM/l)	Plasma Cl^- (mM/l)	Urine Na^+ (mM/l)	Urine K^+ (mM/l)	Urine HCO_3^- (mM/l)	Urine Cl^- (mM/l)	pH	G.F.R. pCO_2 (mmHg)	G.F.R. Vol. (ml/min)	Excretion (ml/min)	Excretion Na^+ (mM/min)	Excretion K^+ (mM/min)	Excretion HCO_3^- (mM/min)	Excretion Cl^- (mM/min)
45 to 30	143.8	2.9	109.0	177.5	72.5	82.1	145.0	7.68	68.00	13.00	689	2.308	0.943	1.067	1.898
20 to 5	143.8	2.9	105.5	195.0	90.0	106.8	155.5	7.72	71	9.47	717	1.847	0.852	1.011	1.470
			5 ml of hydrochlorthiazide (250 mg) intramuscularly.												
0-25	143.8	2.8	104.0	180.0	32.5	41.9	169.5	7.42	65	37.53	690	6.755	1.220	1.573	6.361
0-55	143.8	2.7	105.5	208.8	36.5	47.2	193.5	7.46	65	29.47	663	6.153	1.076	1.391	5.702
0-85	143.8	2.9	101.5	201.3	41.5	60.3	184.5	7.54	65	25.33	643	5.099	1.051	1.510	4.673
00-115	143.8	2.8	104.0	206.3	41.5	45.1	201.5	7.45	68	22.07	673	4.553	0.916	0.995	4.447

DISCUSSION

Results of the survey of urine samples confirmed the findings of other workers (Ashworth & Brody, 1933; Galloway, 1936; Szolnoki, 1941; Poulsen, 1957; Barrada, 1957) that bovine urine is usually alkaline. As, with the exception of those of Szolnoki, all the values quoted had been obtained from urine exposed to air for varying lengths of time, it seems likely that the previously reported values were elevated by CO_2 loss. A recent paper (Woelfel, Calhoun, Rousseau, Eaton & Nielsen, 1963) reported pH values as high as 8.52 from bovine urine collected over 24 hrs in a carboy and mixed thoroughly before measurement.

In the present study, despite high bicarbonate concentrations (254 mM/l.), urinary pH values rarely exceed 8.2. Subsequent work using carbonic anhydrase inhibitors in cattle showed that, despite bicarbonate concentrations greater than 300 mM/l, urinary pH rarely exceeded 8.0 (Anderson & Pickering, 1964).

The mean bicarbonate concentration in all urine samples was 131.0 $\pm$ 58.3 mM/l. Other workers have reported urine bicarbonate values in cattle under abnormal conditions (Brouwer, 1935; Lepard, Page, Maynard, Rasmussen & Savage, 1940; Dale, Goberdahn &

Brody, 1954), but there appears to be no other record of a survey of normal bicarbonate values in bovine urine. Several extensive studies of electrolyte excretion in cattle surprisingly omit bicarbonate measurements (Sellers & Roepke, 1951 a, b, c; Blaxter & Wood, 1953; Vogel, 1962).

There are no other reports of the relationship between pH values and bicarbonate concentration in bovine urine. The curvilinear relationship found in the present work (Fig. 20) is similar to that found in urine samples from the dog (Pitts & Lotspeich, 1946) and man (Pitts et al, 1948) receiving an intravenous infusion of sodium bicarbonate. As in the findings of these workers, the pCO_2 values were found to be greatest at high bicarbonate concentrations and low when bicarbonate concentration was low. The mean pCO_2 (64 mm Hg) was significantly higher than the normal mean pCO_2 of arterial blood (40 mm Hg). The relationship between pH and bicarbonate concentration was not so close as found by Pitts and his co-workers. As these workers obtained their results from a small number of dogs subjected to progressively increasing bicarbonate loading under laboratory conditions, while the present data was obtained from a large number of cattle under field conditions, the wider scatter in

the pH bicarbonate relationship of the bovine samples was not unexpected.

Marshall (1922) stated that the physico-chemical relationship of pH to the 'constant' carbonic acid content of urine ensures that urine pH never rises above 8.0. This concept was based on the fact that urinary bicarbonate concentration greater than 200 mM/l. had not been found, and that equilibration of pCO_2 values must occur between tubular fluid, blood and interstitial fluid. Though Gamble (1922) supported this concept, there is good evidence that the pCO_2 of urine shows marked variations and may greatly exceed that of plasma (Pitts & Lotspeich, 1946; Pitts et al, 1948; Ryberg, 1948; Portwood, Seldin, Rector & Cade, 1959). Two theories have been proposed to explain the high pCO_2 found in alkaline urine. Pitts & Lotspeich (1946) attributed the high pCO_2 of alkaline urine to the secretion of H^+ into tubular fluid containing large quantities of bicarbonate. Delayed dehydration of the carbonic acid thus formed in the tubular lumen results in elevated carbonic acid, and hence pCO_2 values. Kennedy, Orloff & Berliner (1952) on the other hand advanced the theory that the high carbonic acid was the result of the admixture in the renal pelvis of alkaline and acid urines from the hetero-

heterogeneous nephrons. In a critical examination of the latter hypothesis, however, Rector, Portwood & Seldin (1959) concluded that it could not account for the high pCO_2 values found in their experiments, and that the theory suggested by Pitts was the most likely explanation of the phenomenon.

This theory, derived from experiments on alkalotic dogs may explain the high pCO_2 values of bovine urine. In the maintenance of acid-base equilibrium, the cow excretes the excess potassium in the diet with bicarbonate in the urine. Bicarbonate is, however, reabsorbed from the renal tubules and if the mechanism is similar to that which is presently accepted in man (Pitts, 1963), then H^+ is continually secreted into the tubular fluid rich in bicarbonate, thus creating a high U/P carbonic acid ratio.

In a survey of the concentrations of a urinary constituent, the range found is inevitably wide as a result not only of variations in acid-base balance, but also of differing degrees of hydration and water intake in individuals. Barclay & Nutt (1944) reported that, in human subjects, in which there were marked individual variations in water intake, diuresis caused a fall in urinary pH when the initial value was above 6.9, and a rise when the initial value was below 6.9. The increase

in the pH of acid urine which accompanies diuresis was found to result from a decrease in the secretion of hydrogen ions rather than a physico-chemical change accompanying dilution of phosphate buffers (Nutbourne & de Wardener, 1960). Individual variabilities of water intake should not, however, be pronounced in grazing cattle, in that there is a high proportion of water to dry matter in the diet, and there is therefore, a high obligatory intake particularly in lush wet pasture. Despite constant dietary and environmental conditions, however, marked individual variations in electrolyte excretion patterns have been noted in ruminants (Dobson & Phillipson, 1962).

The results of the survey indicated that under the conditions described, the urine was invariably alkaline, suggesting that the role of the kidney was to combat the onset of a potential alkalosis.

Plasma concentration and urinary output of total CO_2

The procedure of sampling arterial blood from the brachial artery of the cow may result in marked diuresis (Author, 1961). Thus, while it is desirable that a blood sample be taken in each clearance period, the disturbance to the animal of repeated arterial puncture is undesirable. Fisher (1961) in a study of hourly

variations in total CO_2 of arterial plasma in dairy cattle found that the maximum variation was 1.7 mM/l. Initial studies in the present work confirmed these findings. These findings, and the reduction in disturbance to the animal justified the use of a single arterial sample to provide total CO_2 values for several clearance periods.

The mean total CO_2 concentration of arterial plasma from the 12 cows was 29.1 $\pm$ 2.2 mM/l. Fisher (1959) found a mean value of 26.9 $\pm$ 2.8 mM/l. in 141 Ayrshire dairy cows, and observed no significant variations attributable to pregnancy, lactation or season. The cattle sampled by Fisher were on a similar diet and management to those used in the present study. Aalund & Nielsen (1960) determined standard bicarbonate by the method of Astrup & Jørgensen (1957) on venous blood from 116 cows at the slaughter-house. They found a range of values from 17.0 to 27.0 mM/l. In the absence of further data, it appears from their histogram that most values were between 22 and 25 mM/l. Though data on standard bicarbonate is not strictly comparable with manometric determinations of total CO_2 values, there is no doubt that these values are very much lower than the present results or those of Fisher. Though their animals were described as clinically

normal, their presence in the slaughter-house was unlikely to have been conducive to physiological conditions.

In subsequent work the mean carbonic acid concentration of bovine arterial blood samples was found to be 1.3 mM/l. By subtracting this value from the mean total CO_2 concentration the mean bicarbonate concentration of the 12 cows was calculated to be 27.8 ± 2.2 mM/l. Fisher (1959) calculated from his results that bicarbonate concentration of bovine arterial plasma was 25.8 mM/l. There are few records of normal arterial bicarbonate concentrations in man, and Pitts, a foremost authority, has quoted several values; 23 - 26 mM/l. (1945), 24 - 28 mM/l. (1949), and 26 - 28 mM/l. (1963). This latter range Pitts (1963) described as the so-called 'bicarbonate renal threshhold' in man. He stated that under normal conditions the plasma concentration of bicarbonate is poised at a value slightly below the renal threshhold, at which concentration bicarbonate is totally reabsorbed from the filtrate and the urine is acid and bicarbonate-free.

In the present study, urine was alkaline and contained appreciable bicarbonate concentrations when plasma bicarbonate ranged from 24.3 - 32.4 mM/l. Thus the so-called bicarbonate renal threshhold is

below 24.3 mM/l. in the cow.

The lowest plasma total CO_2 concentration found by Fisher (1959) was 22.3 mM/l. (approx. 21.0 mM/l. of bicarbonate), and subsequent work by the present author showed that bicarbonate was still excreted in the urine at this concentration (p.152). The appearance of bicarbonate in the urine at the lowest limits of normal plasma concentration in the cow suggests that the ability of this species to conserve bicarbonate may be poorly developed in comparison to man and the dog, and merits further study.

The absence of any relationship between plasma bicarbonate concentration and the rate of bicarbonate excretion (Fig. 27) suggested that excretion rate was related to some other factor than to plasma bicarbonate levels alone. The participation of factors other than plasma bicarbonate concentration is evident in one cow (Table 32, Case no. 10150) in which bicarbonate excretion was very low despite a normal plasma concentration.

Study of the effect of acute changes in urine flow on bicarbonate excretion was made possible by the diuretic response to arterial puncture. Fig. 23 shows that there is a trend for bicarbonate excretion to be greatest at high rates of urine flow. This is in

general agreement with the postulate of Nutbourne & de Wardener (1960) that diuresis causes a fall in the excretion of titratable acid. Their experiments were carried out on man excreting an acid urine in which they found a rise in pH and fall in titratable acid excretion greater than could be accounted for by dilution effects. The interdependence of H^+ secretion and bicarbonate reabsorption (Fig. 19) is such that a fall in H^+ secretion is likely to be associated with a fall in bicarbonate reabsorption.

The rate of filtration, reabsorption and excretion of bicarbonate. Two alterations in technique adopted in these studies resulted in a marked improvement of the reliability of bicarbonate clearance studies. Firstly, the efficiency of urine collection was increased by use of the Ramshorn catheter, and secondly, sampling of arterial blood from the coccygeal artery was not accompanied by the diuretic response which was previously evoked by puncture of the brachial artery.

There are few descriptions of arterial blood sampling in cattle. The author initially followed the method described by Fisher (1956) in puncture of the brachial artery, but the painful stimulus of probing unanaesthetised tissues caused the diuretic

phenomena described. Blackwood & Stirling (1932) mention puncture of the radial artery in the cow without giving details, except to state that 'the procedure is attended by considerable disturbance to the animal'. Serial blood sampling from the exteriorised carotid artery of calves was abandoned by Bianca & Findlay (1962) as being unsatisfactory. A description by Saarinen (1938) is the only available record of arterial blood sampling from the coccygeal artery of the cow. The advantage of this technique in the present work was that as a result of posterior epidural anaesthesia induced prior to urethral catheterisation for the purpose of abolishing discomfort and micturition reflexes, the tail was fully anaesthetised for several hours. Puncture of the artery was therefore a painless procedure and was not accompanied by the side-effects noted in puncture of the brachial artery. The disadvantage of the technique was that puncture was often followed by development of a haematoma which limited the number of subsequent samples which could be taken.

The fact that the reabsorption rate of bicarbonate was proportional to the plasma concentration over the range of plasma concentrations encountered (21.9 - 32.8) mM/l. may indicate that within this range, reabsorption

rate never reached a maximum level and that further increase of plasma bicarbonate concentration beyond this range would have resulted in a further increase in reabsorption rate. The data does not, however, preclude the possibility that the reabsorption rates found in each animal represented the characteristic maximum reabsorption rate in that animal. This second interpretation is, however, the less likely as in case no. 12916 (Table 33), 98% of the filtered bicarbonate was reabsorbed despite a high plasma bicarbonate concentration. If this reabsorption rate had been maximal it is likely that the rate of bicarbonate excretion would have been a greater percentage of the rate of filtration (Pitts & Lotspeich, 1946). It is concluded therefore that the maximal reabsorption rate in one cow (12916) was greater than 3.21 mM/100 ml. of glomerular filtrate and that reabsorption rate was proportional to plasma concentration at all plasma levels between 21.9 and 32.8 mM/l. In Fig. 25 the regression of reabsorption rate on plasma concentration intersects with the broken line representing 100% reabsorption at a plasma concentration of 20 mM/l, thus suggesting that at this plasma concentration and below it, total reabsorption of bicarbonate by the tubules would result in the excretion of a bicarbonate-free

acid urine. The lowest total CO_2 concentration in arterial plasma found by Fisher (1959) in 141 normal dairy cows was 22.3 mM/l, thus it appears that normal plasma levels rarely fall low enough to allow 100% reabsorption.

Reabsorption rate is expressed as ml/100 ml. of glomerular filtrate throughout this report for two reasons; firstly, to eliminate variations between animals with different glomerular filtration rates, and secondly because Pitts & Lotspeich (1946) showed that in the dog, the quantity of bicarbonate reabsorbed per unit of time varies directly and nearly proportionally with changes in the rate of glomerular filtration. In the present work, no attempt was made to vary glomerular filtration rate, and the normal variations found in the individual animal were not great enough to show a significant relationship between filtration rate and reabsorption rate. The over-all results of the 3 cows did show a significant relationship between filtration rate and reabsorption rate, but this was largely due to the fact that an animal with a high filtration rate had a higherreabsorptive capacity than an animal with a lower filtration rate. Pitts (1963) pointed out that this relationship of bicarbonate reabsorption rate to filtration rate is particularly important to the dog

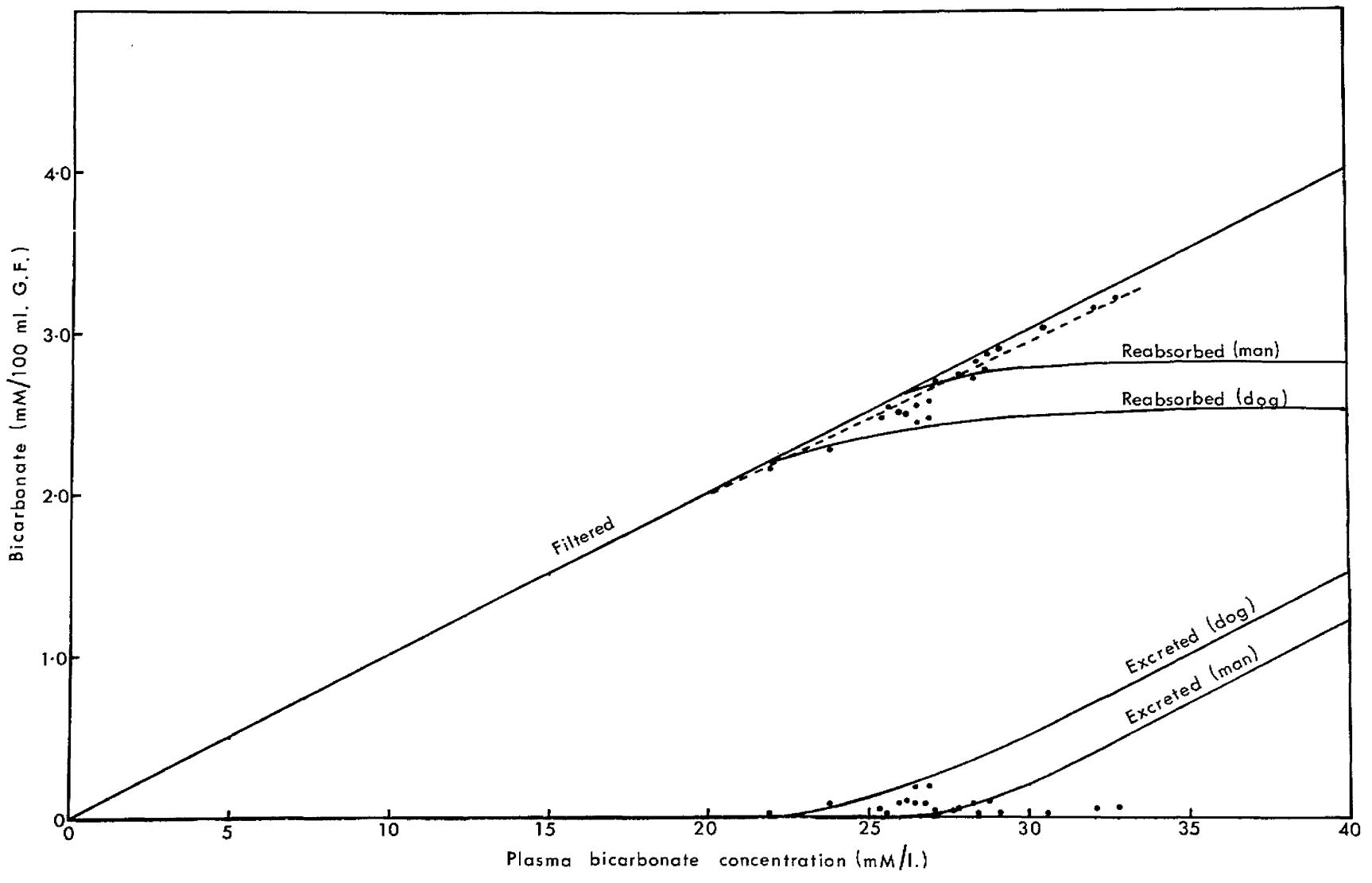
which has a labile glomerular filtration rate. If bicarbonate were reabsorbed by the same type of mechanism as exists for, say, glucose, in which the tubular maximum is limited by time, then the marked rise in filtration rate which follows a meal rich in protein would result in bicarbonate depletion due to excess urinary losses. Any increase in filtration rate is however accompanied by an increase in bicarbonate reabsorption rate, and thus homeostasis is maintained.

The mean rate of bicarbonate reabsorption found in three cows was 2.63 mM/100 ml. of glomerular filtrate. To allow comparison with values in man and the dog, the data from the three species is shown on Fig. 35. The values from the present work on cattle is superimposed on the findings of Pitts & Lotspeich (1946) in the dog, and Pitts et al (1949) in man.

It can be seen that even at the highest plasma bicarbonate concentrations, the cow excretes a relatively small proportion of the filtered load of bicarbonate, whereas at a corresponding plasma concentration a much larger proportion of the filtered load is excreted by the dog. The difference between the cow and man is less striking.

At the lower limits of the plasma concentration, the similarity between the cow and the dog is greater

Fig. 35. The relationship of the filtration, reabsorption and excretion of bicarbonate to the bicarbonate concentration in arterial plasma in man, dog and cow. The data for man and the dog was obtained respectively from Pitts, Ayer and Schiess (1949) and Pitts and Lotspeich (1946). The closed circles mark the relationship found in cattle, and the broken line is the regression of bicarbonate reabsorption per 100 ml. glomerular filtrate on plasma bicarbonate concentration.


Bicarbonate (mM/100 ml. G.F.)
4·0
3·0
2·0
1·0
0
0
5
10
15
20
25
30
35
40
Plasma bicarbonate concentration (mM/l.)
Filtered
Reabsorbed (man)
Reabsorbed (dog)
Excreted (dog)
Excreted (man)

than between the cow and man. Bicarbonate was excreted by the cow at all plasma levels down to 22 mM/l. In the dog, bicarbonate excretion begins to occur between 20 and 25 mM/l, whereas in man, little or no excretion occurs at plasma levels below 25 mM/l.

The results of the present work indicated that in the three cows studied, bicarbonate was excreted in the urine at plasma concentrations between 22 and 33 mM/l. Bicarbonate reabsorption rate was found to be higher than the maximal rate in the dog and at the highest reabsorption rate, (3.2 mM/100 ml. of glomerular filtrate) there was no evidence that the maximum had been reached. Bicarbonate excretion remained at a proportionately much lower level at high plasma concentrations than in man and the dog. Thus the threshhold for bicarbonate excretion appears to be lower, or less well defined than in man, while reabsorption rate appears to be capable of increasing above the maximal rate reported in man and the dog.

There are four main factors which influence bicarbonate reabsorption. Some evidence is available concerning the participation of these factors in reabsorption rates in the cow.

Partial pressure of CO_2 in arterial blood It is well

known that the maximum reabsorption rate of bicarbonate can be increased by elevation of blood pCO_2 (Relman, Etsten & Schwartz, 1953; Schwartz, Falbriard & Lemieux, 1959). Thus when exposed to progressively increasing concentrations of CO_2 in inspired air, the bicarbonate reabsorption rate of anaesthetised dogs displayed characteristics which are similar to the present findings, i.e. that when plasma pCO_2 was about 100 mm Hg, reabsorption rate rose with increasing plasma concentration until a maximal reabsorption rate of 3.8 mM/100 ml. of glomerular filtrate was reached. If, therefore, pCO_2 values in the arterial blood of the cow were different from those in man and the dog, then this would contribute the understanding of bicarbonate reabsorption in this species.

There are few records of normal pCO_2 values from arterial blood in cattle, but Hansson & Johannesson (1958) recorded pCO_2 values from aortic blood in the cow immediately before anaesthesia, and found these to be similar to normal values in man.

In the present study the pCO_2 of coccygeal arterial blood was found in each sample and the mean value of 44 mm Hg was similar to the values obtained by Hansson and within the range of normal values in man (Searcy, Gordon & Simms, 1963). It does not, therefore, appear

that the arterial pCO_2 of the cow contributes substantially to the pattern of bicarbonate reabsorption under normal conditions.

Plasma chloride concentration Pitts & Lotspeich (1946) showed that a reciprocal relationship existed between the excretion of bicarbonate and chloride. The effect of this mechanism was to maintain at a constant level the sum of the bicarbonate and chloride concentrations. Thus an increase in the concentration of either of these anions resulted in lowering of the renal threshhold for the other.

In section 4 in which chloride concentration was measured in all blood samples, it was found that plasma chloride values in the cow are not different from the normal values in man. No experiments on bicarbonate clearance have been carried out during alteration of plasma chloride concentrations, and until this is done it remains to be seen whether the bicarbonate chloride inter-relationship is the same in the cow as in man and the dog.

Variations in the body store of potassium The relationship between the ingestion or administration of potassium salts and the mechanisms controlling the reabsorption

and excretion of bicarbonate has been known for some years. Loeb et al (1932) noted that the urine became alkaline after ingestion of potassium chloride by human subjects, and subsequent study has shown that infusion of potassium chloride caused a progressive fall in plasma bicarbonate which was partly attributable to a decrease in the rate of bicarbonate reabsorption (Roberts, Magida & Pitts, 1953) and partly to the diffusion of bicarbonate into cells (Bourdillon, 1937). Roberts et al (1953) confirmed the earlier concept of Berliner, Kennedy & Orloff (1951) that competition existed between hydrogen and potassium for exchange with sodium in the distal nephron, and thus provided a logical explanation for the inter-relationship between potassium ingestion and bicarbonate excretion. When Fuller, MacLeod & Pitts (1955) fed potassium chloride enriched diets to dogs for several days, there followed a much greater potassium excretion than they attained in acute experiments, and no alteration in bicarbonate output. They concluded that bicarbonate reabsorption was related to the plasma concentration of potassium and was unaffected by the large changes in potassium excretion which followed potassium feeding. It has been shown by the author that the response of the normal cow to an intravenous load of potassium chloride

(Anderson & Pickering, 1962) is similar to that shown by the dog which has previously been made 'potassium tolorant' by feeding potassium salts for two weeks before infusion. The fact that no relationship was found between the rate of bicarbonate excretion and the plasma bicarbonate concentration in the present work suggests the possibility that bicarbonate excretion in the cow over the normal plasma range may be linked to variations in plasma potassium as was found by Fuller et al (1955) rather than to plasma bicarbonate concentration.

The cows normally potassium-rich diet is not, however, comparable to the diet of experimental dogs to which potassium chloride has been added, as the potassium which predominates in the herbivores diet is largely associated with organic anions. Such a diet presents fewer problems to the bovine kidney than does a diet of acid ash to the human and canine kidney. In the normal cow there is less requirement for the urinary acidifying mechanisms which exist in man and the dog, as the contribution of the bovine kidney to homeostasis is the prevention of a potential alkalosis rather than an acidosis. The question of mechanisms and efficiency of the bovine kidney in the maintenance of homeostasis in the face of dietary and disease requirements for the

excretion of an acid urine is at present unanswered. It is clear, however, that if a similar competition for the tubular secretion of potassium and hydrogen ion exists in the cow as in man and the dog, then the continual requirement for the excretion of potassium must limit the herbivores ability to excrete hydrogen ion.

Effect of acetazolamide In all experiments, the intravenous administration of acetazolamide resulted in a prompt increase in bicarbonate excretion. Both the increase in the rate of urine flow, and the increase in urinary bicarbonate concentration contributed to the increase in excretion. Bicarbonate excretion was accompanied by an increase in sodium excretion in all experiments, but in 3 experiments there was also a marked (x 2) increase in potassium excretion (Tables 36, 40, 43). Chloride excretion was slightly increased in all experiments.

This response of the cow to the administration of a carbonic anhydrase inhibitor was similar to that seen in man and the dog in so far as sodium and bicarbonate excretion was enhanced, urinary pH was elevated and there was a pronounced increase in urine flow.

Fuller et al (1955) found that after reduction of bicarbonate reabsorption by administration of acetazo-

acetazolamide, no further reduction could be induced by infusion of potassium salts. In the present experiments the relatively high potassium intake of the cow and its alkaline urine rich in bicarbonate did not prevent a clear response to acetazolamide which was substantially the same as that which occurs in man and the dog. This suggested that carbonic anhydrase plays a similar central role in the renal control of electrolyte excretion in the cow as in other animals. Thus, despite the alkalinity of bovine urine it is probable that the reabsorption of sodium and bicarbonate from the tubular fluid was dependent on the secretion of hydrogen ions made available by the hydration of CO_2 in the presence of carbonic anhydrase.

The anomalous finding of a high urinary pCO_2 after carbonic anhydrase inhibition in the present study has been reported in the dog by other workers (Portwood et al, 1959).

The normally high pCO_2 values found in alkaline urines have been discussed elsewhere (p.[163]). The generally accepted explanation is that of the delayed intra-luminal dehydration of the carbonic acid formed by the secretion of H^+ ions into a bicarbonate rich fluid. Pitts & Ochwadt (1956) supported this hypothesis by showing that infusion of carbonic anhydrase into

alkalotic dogs abolished the high urinary pCO_2 values by accelerating the intra-luminal dehydration of carbonic acid. It might have been expected that a carbonic anhydrase inhibitor would have exerted a similar effect by decreasing the rate of secretion of hydrogen ions, and thus the rate of formation of carbonic acid. It appears, however, that despite the inhibition of carbonic anhydrase and the consequent decrease in the rate of hydrogen ion secretion, there is a sufficient supply of hydrogen ions from sources independent of carbonic anhydrase to elevate the pCO_2 of alkaline urine above the pre-dosing levels (Rector et al, 1960).

The relatively weak kaliuretic action of acetazolamide in cattle observed in the present work differed from its reported action in the dog (Berliner et al, 1951) and in man (Counihan, Evans & Milne, 1954). This difference can be explained on the basis of existing knowledge of ionic exchange in the renal tubules. It is known that there is competition between hydrogen and potassium for some component of the ion exchange mechanism by which both are secreted (Berliner et al, 1951). Thus, when the secretion of hydrogen ion is inhibited in the dog, which is normally producing an acid urine, the secretion of potassium ions

is greatly enhanced. When the secretion of hydrogen ion is already low, as in the cow producing an alkaline urine, then the effect of further suppression by inhibition of carbonic anhydrase is minimised. It is not unexpected, therefore, that the kaliuretic effect of acetazolamide should be less marked in the cow than in man and the dog. It is noteworthy that in the only experiments in which a marked kaliuresis occurred, the pre-dosing rate of potassium excretion was low (Tables 36, 40, 43).

It is beyond the scope of the present work to discuss in detail the other changes which resulted from acetazolamide administration. The reduction in glomerular filtration rate which followed administration of acetazolamide has been noted by other workers (Berliner et al, 1951; Berliner & Orloff, 1956) and has been attributed to a fall in the extracellular fluid volume after acetazolamide. In the course of the present work serial measurements of inulin space were made during each clearance period, and no consistent tendency for a fall in extracellular fluid volume was noted.

The results of the experiments with hydrochlorothiazide confirmed that it was an effective diuretic in cattle. There was a sharp rise in the rate of

sodium and chloride excretion, but at the dosages used, there was little carbonic anhydrase inhibiting activity. Hydrochlorothiazide did not, therefore, alter the rate of bicarbonate excretion, nor significantly affect urinary pH. It is likely that at a higher dose rate a carbonic anhydrase inhibiting effect would have occurred as has been noted in other species (Beyer & Baer, 1961).

S U M M A R Y A N D C O N C L U S I O N S

1. The absence of any quantitative studies to support the generally held belief in the causal relationship between the 'alkaline ash' diet of the cow, and the excretion of an alkaline urine, prompted the present study on the excretion of bicarbonate by the dairy cow.

2. The literature relating to the study of renal function, the investigation of the renal control of acid-base balance, and the application of clearance studies to cattle has been reviewed.

3. The factors affecting the collection and storage of bovine urine samples for the estimation of pH and bicarbonate concentration have been examined. The change in pH and total CO_2 of samples exposed to air was shown, and a method of anaerobic collection described.

4. The diuretic response of cows to a painful stimulus has been recorded. The effect on urinary bicarbonate concentration and excretion rate was examined, and these results compared with the renal responses of other species to painful stimuli.

5. Observations on catheterisation of the bovine

bladder without anaesthesia, with topical anaesthesia, and with epidural anaesthesia have been made. The advantages of epidural anaesthesia for renal clearance studies in the cow have been indicated and discussed.

6. The normal pressure of the bovine bladder was shown to be sub-atmospheric. Pressure values in newly emptied bladder were between -5 and -10 cms of water. The effect of intra-abdominal pressure changes and the response of the bladder to filling were recorded. It was concluded that the normally sub-atmospheric pressure of the bladder was related to a negative intra-abdominal pressure.

7. The 'first appearance time' in cattle was found by injection of a solution of phenol red into the jugular vein during continuous urine collection. The mean delay between injection of the dye and its first appearance in the collection tubing was 162 secs.

8. The methods of urine collection used in the cow have been described. The techniques used to assess the accuracy of urine collection have been described, and the results of their application to the methods used in the present study recorded. The advantages of continuous perfusion of the bladder during clearance studies were discussed.

9. The measurement of inulin clearance in the cow by

a single injection method was investigated. The results of clearance measurements on five cows in 10 experiments were recorded. Inulin space values were calculated at 27 min. and 97 min. in each experiment in the course of the clearance measurement. It was concluded that there is little justification for the measurement of extra cellular space in cattle after a single injection of inulin. The single injection method of measuring inulin clearance was found to have poor repeatability, and was only suitable for a single determination of glomerular filtration rate.

10. The measurement of inulin clearance in the cow by constant infusion was investigated. In 103 determinations on seven cows, the glomerular filtration rate (inulin clearance) was 1,100 $\pm$ 236 ml./min/500 kg. Some further evidence was presented for the validity of inulin clearance as a measure of glomerular filtration rate in the cow. Day to day variations in the filtration rate of the same individual were recorded. The repeatability of the method described was found to be within the acceptable limits of accuracy for clearance studies.

11. A survey of urinary pH and bicarbonate values of urine samples from a herd of Ayrshire dairy cows under indoor conditions in the winter, and at grass in the

summer, was carried out. The mean bicarbonate concentration was 131.0 ± 58.3 mM/l. and the mean pH was 7.92 ± 0.21. There was no significant difference between the values obtained when at grass, or indoors, nor was there a significant difference between groups of lactating and non-lactating, and pregnant and non-pregnant animals. The mean calculated pCO_2 of all samples was 64 ± 22 mm Hg. The relationships between pH, pCO_2, and bicarbonate concentration were the same as that observed in dogs subjected to experimental sodium bicarbonate infusion.

12. Arterial blood was sampled anaerobically during urine collection in 60 experiments on 12 cows. The mean total CO_2 concentration in the plasma was 29.1 ± 2.2 mM/l. (Range 26.2 - 33.0) and the mean excretion rate of total CO_2 was 1.38 ± 0.73 mM/min (Range 0.05 - 4.28). There was no over-all relationship between plasma total CO_2 concentration and the urinary excretion of total CO_2.

13. Bicarbonate clearance measurements were carried out during inulin infusion in 20 experiments on three cows. The mean rate of bicarbonate reabsorption in each cow was (2.90 ± 0.19, 2.67 ± 0.15, and 2.39 ± 0.12) mM/100 ml. glomerular filtrate, while the corresponding arterial plasma bicarbonate concentrations were (29.4 ±

(29.4 ± 2.1, 27.2 ± 1.1 and 25.0 ± 1.8) mM/l. There was a significant proportional relationship between the plasma concentration and reabsorption rate. The mean arterial pCO_2 was 44 (Range 43 - 45) mm Hg in the three cows. Bicarbonate excretion rate was (0.039 ± 0.029, 0.113 ± 0.049 and 0.110 ± 0.067) mM/100 ml. glomerular filtrate, and was not related to plasma bicarbonate concentration.

There was, therefore, a bicarbonate 'leak' into the urine at all plasma concentrations between 21.9 and 32.8 mM/l.

14. The excretion of sodium and bicarbonate was markedly enhanced by a carbonic anhydrase inhibitor, acetazolamide. A less marked kaliuresis occurred in the cow than has been observed in man and the dog. A marked fall in glomerular filtration rate occurred after administration of acetazolamide. Hydrochlorothiazide caused a marked natriuresis and chloruresis but did not show a carbonic anhydrase inhibiting effect at the dosage used. Both drugs caused a marked increase in the rate of urine flow.

15. The following conclusions may be drawn from the results of the foregoing study.

As the mean bicarbonate concentration of arterial plasma in the cow (27.5 mM/l.) is close to the upper

limit of the normal range in man (26 - 28) mM/l. (Pitts, 1963), the invariable presence of bicarbonate in bovine urine is partly related to a high plasma concentration. A marked bicarbonate 'leak' occurs into bovine urine, however, at all plasma levels between 22 and 33 mM/l, while in man bicarbonate is virtually absent from the urine at plasma levels below 25 mM/l.

Of the factors known to alter the inter-relationship between plasma bicarbonate concentration, and renal reabsorption, and excretion, it appears that the most likely explanation for the constant presence of bicarbonate in bovine urine is the high rate of potassium intake, and excretion.

The author believes that further investigation of the renal control of acid-base balance in the cow along the line begun in the present study will have an important bearing on the understanding and treatment of metabolic diseases in cattle.

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