

THE RENAL RESPONSE OF PUPPIES TO AN ACIDOSIS

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If chloride or sulphate or any other strong non-volatile anion is introduced into the body, unaccompanied by an equivalent amount of fixed base, hydrogen ions become associated with the anions and the *status quo* can only be restored by the excretion of the hydrogen ions and also the Cl or SO₄ ions. Pitts (1950), Gamble (1952), and many others, have described these processes in considerable detail in ways best suited to their problems or their lines of thought.

Infants, particularly premature infants, are well known to be very liable to suffer from an acidosis (Gordon, McNamara & Benjamin, 1948) and to recover from one rather slowly, but curiously little research has been devoted to the reasons for this. A summary of the knowledge available at that time was given by McCance (1950). Little has been gained since, but it is clear that the failure of infants to correct an acidosis so rapidly and completely as adults may be due to one or more of the following:

(a) An unresponsive respiratory centre or inefficient mechanisms in the lungs for excreting CO₂. With these aspects the present article is not concerned.

(b) A failure to lower the pH of the urine or insufficient buffer substances in the urine to allow H ions to be excreted in any quantity within the pH limits of the urine. Dean & McCance (1948) suggested that the latter might be important when they found so little phosphate in the urine of normal breast-fed infants.

(c) A limited capacity to excrete the anions. This has been hinted at by the work on infantile glomerular filtration rates, and the tendency of infants to 'retain' administered sodium chloride has been recognized for many years (Ohlmann, 1920). McCance & Young (1941) found lower chloride clearances in infants, particularly premature infants, than in adults per unit of surface area or of body weight.

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(d) Undeveloped mechanisms for NH_3 formation. This was investigated in human infants by Gordon *et al.* (1948). These authors also studied the excretion of sodium, potassium and in some instances of phosphorus, but they did not themselves study any adults, and their infants were of variable age. They concluded that the mechanism for the formation of NH_3 was often undeveloped in very young infants, but that the bad effects of an acidosis were sometimes due to a peculiar systemic susceptibility. The ammonia mechanisms have also been investigated *in vitro* at two levels of pH by the tissue-slice technique (Robinson, 1954) with and without added glutamine (Hines & McCance, 1954). The kidneys of new-born rats and dogs made less ammonia than those of adults.

The present work was undertaken in order to compare the renal responses of mature and new-born animals to an acidosis, and for this purpose to examine particularly mechanisms *b*, *c* and *d* which have been outlined above. Dogs have been used and have proved very suitable animals for this work.

MATERIAL AND METHODS

Three adult bitches and sixteen of their puppies, all thoroughbred bull terriers, have been used. Elimination of urine and faeces in suckling puppies is normally brought about reflexly by the mother licking the perineum. Uncontaminated urine may be obtained quantitatively by tickling the perineal area of the puppy and collecting the urine in a small tube. The reflex becomes more difficult to elicit as the puppy gets older, and impossible after 9 or 10 weeks. Owing to the difficulties of dealing with very small volumes of urine, and the impossibility of allowing the puppies to feed from the mother and at the same time to obtain all of the urine formed by them, the experiments have been of short duration, and, after the control period, enough fluid was administered to provide a diuresis.

Litter-mates of mixed sex of various ages, but mostly 2 days old, were removed from the mother 3 or 4 hr before the experiments were due to begin. They were placed in a warmed box in individual compartments to prevent them from licking each other and so initiating the elimination reflex. After their bladders had been emptied they were left for 1 hr and their bladders emptied again. Two per cent of the body weights of sterile $\text{m}/6\text{-NH}_4\text{Cl}$ or $\text{m}/12\text{-(NH}_4)_2\text{SO}_4$ or isotonic (0.9%) NaCl at 37° C was then given intraperitoneally, and 3% of the body weight of water was administered by stomach tube within a space of 3 or 4 min. $\text{m}/12\text{-(NH}_4)_2\text{SO}_4$ is slightly hypotonic, but contains the same equivalence of acid per unit volume as isotonic NH_4Cl . The total acid administered was 3.3 m.equiv/kg of body weight. The urines were collected at hourly intervals for 6–8 hr. Blood was then withdrawn under paraffin by cardiac puncture, and the puppies were returned to the mother.

The adult bitches were trained for several months, and then subjected to a similar procedure except that: (a) drinking water was removed 10–12 hr before the experiments began; (b) 15–20 min were allowed for the administration of the water by stomach tube; (c) urine was collected continuously from an indwelling gum elastic catheter; (d) blood was taken under paraffin from a subcutaneous branch of the saphenous vein at the end of the period of measurement, which usually lasted 5–6 hr.

In several cases, blood was withdrawn from both puppies and adults at earlier stages in order to determine the extent of the internal acidosis, but these animals were not used for analysing the renal response to the acidosis because the emotional 'stress' of removing blood may be considerable in young children (Barnett, Vesterdal, McNamara & Lauson, 1952; McCance, Naylor & Widdowson, 1954) and is unavoidable in puppies. Verney (1946) has described the effects in dogs.

Na and K were determined by the Beckman Flame Photometer with model DU spectrophotometer. Both urines and sera were usually diluted 1:100 for this purpose. Sendroy's (1937) iodate

method was used for estimating Cl; and Conway's (1950) micro-diffusion method for NH_3 . The NH_3 was liberated with saturated K_2CO_3 and absorbed into a boric acid solution. Both sera and urines were deproteinized with trichloroacetic acid for the determination of creatinine and inorganic phosphate. A slight modification in the method described by Barrett & Addis (1947) was used for creatinine, and the method of Fiske & Subbarow (1925) for phosphate. pH was measured at room temperature by the macro-Cambridge glass electrode, or by a Beckman micro-capillary tube electrode attached to a Cambridge potentiometer. The figures were 'corrected' to 37° C.

RESULTS

The effect of a diuresis on the pH of the urine

When experiments are carried out in the manner described, both dogs and puppies respond to the administration of the fluids by a rapid increase in the volume of urine excreted. The time relationship of this and its effect on the pH of the urine in dogs was investigated by giving 2% of the body weight of isotonic NaCl intraperitoneally and 3% of the body weight of water by mouth, and the results are shown in Fig. 1. The pH of the urine rose and fell quite

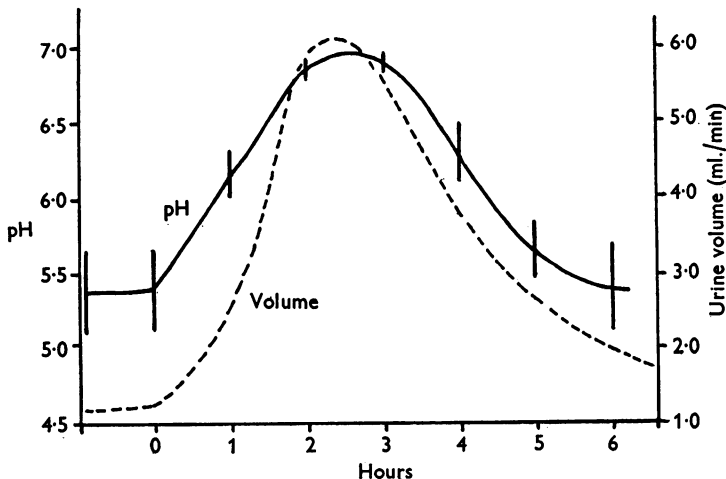


Fig. 1. The effect of a diuresis on the pH of the urine in dogs. The vertical lines indicate standard deviations.

regularly with the increase and decrease in the flow of urine. Eggleton (1947) found a similar change in pH to result from a diuresis in adult man, and McCance & von Finck (1947) from a diuresis in two human infants and one adult. As will be shown later, this change complicates some of the results which have been obtained after the administration of acid drugs, and it is, therefore, important to recognize it.

The changes in the serum

Table 1 shows the changes in pH and in Cl which were observed in the serum of dogs and puppies after the administration of NH_4Cl and $(\text{NH}_4)_2\text{SO}_4$. The

figures are the average findings from three dogs and five puppies. It will be seen that the changes took place equally rapidly and were, on the whole, greater and more prolonged in the puppies. There is no question, therefore,

TABLE 1. Average findings in the serum (with standard errors of the mean) after the administration of acidifying salts. (a) pH after NH_4Cl and $(\text{NH}_4)_2\text{SO}_4$; (b) Cl as m.equiv/l. after NH_4Cl

| | pH* | | Cl (m.equiv/l.) | |
|---------|-----|-------|-----------------|-----------|
| | Dog | Puppy | Dog | Puppy |
| Initial | 7.4 | 7.4 | 100 ± 3.5 | 105 ± 2.8 |
| 30 min | 7.1 | 7.0 | 115 ± 3.1 | 115 ± 2.1 |
| 2 hr | 7.1 | 7.1 | 106 ± 3.8 | 110 ± 3.1 |
| 6 hr | 7.4 | 7.2 | 99 ± 4.2 | 108 ± 4.0 |

* The standard error of the mean was in all cases $< \pm 0.01$.

that the puppies must have been subjected to an acidosis quite as intense and severe as the dogs. There were no changes in the serum Na, K, or PO_4 , and the findings for these ions have not therefore been given. The concentration of HCO_3 in the serum was not measured, but it may be assumed to have changed in a direction opposite to that of Cl.

The excretion of water and creatinine after the administration of acidifying salts

Fig. 2 shows the percentage of the dose of water which had been excreted by each hour after the two salts had been given to the puppies and the dogs. The

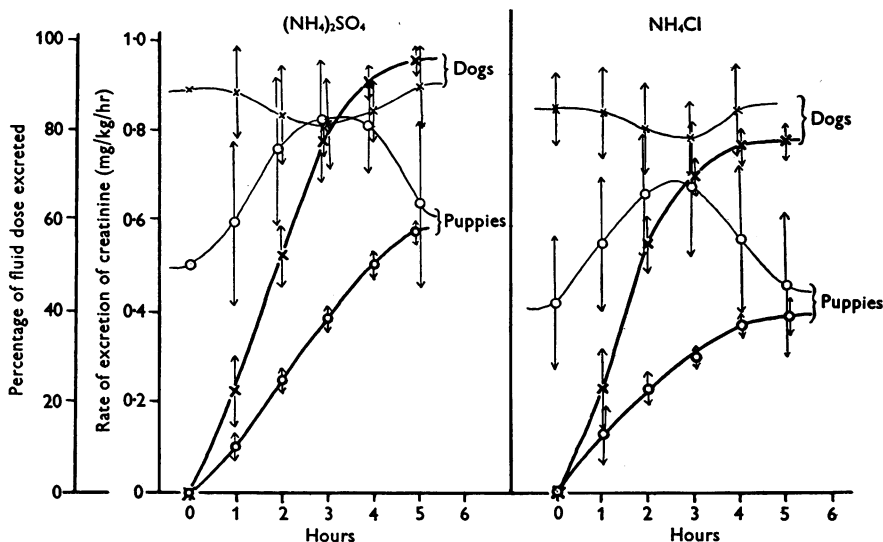


Fig. 2. The excretion of water and the rate of excretion of endogenous creatinine after the administration of $(\text{NH}_4)_2\text{SO}_4$ and NH_4Cl to dogs and puppies. Thin lines creatinine; thick lines water; vertical arrows standard deviations.

slope of the curve gives a measure of the rate. The figure also shows the rate of excretion of endogenous creatinine/kg/hr throughout the experiment. This

may be taken as a measure of changes taking place in the glomerular filtration rate over the 6 hr. It will be observed that: (a) The $(\text{NH}_4)_2\text{SO}_4$ produced a greater diuresis than the NH_4Cl at both ages, but that at both ages and after both salts the puppies excreted less of the administered water than the dogs. The extrarenal losses were not measured but the differences are not thought to have been due to them (Adolph, 1943; Heller, 1951; Ames, 1953; McCance *et al.* 1954). This failure to excrete water as rapidly and completely as adults is common to all new-born animals so far investigated. (b) The puppies *increased* their rate of excretion of creatinine while the rate of excretion of water was high. The dogs did not do this and may even have decreased their rate of excretion of creatinine (McCance & Widdowson, unpublished). The difference suggests that a rise in the glomerular filtration rate is an element to be considered in a diuresis in puppies but certainly not in dogs.

The pH of the urine after acidifying salts

Fig. 3 shows the changes in the pH of the urine of puppies and dogs after the administration of water and acidifying salts. The initial rise in pH which was shown by both puppies and dogs may be attributed to the increased flow

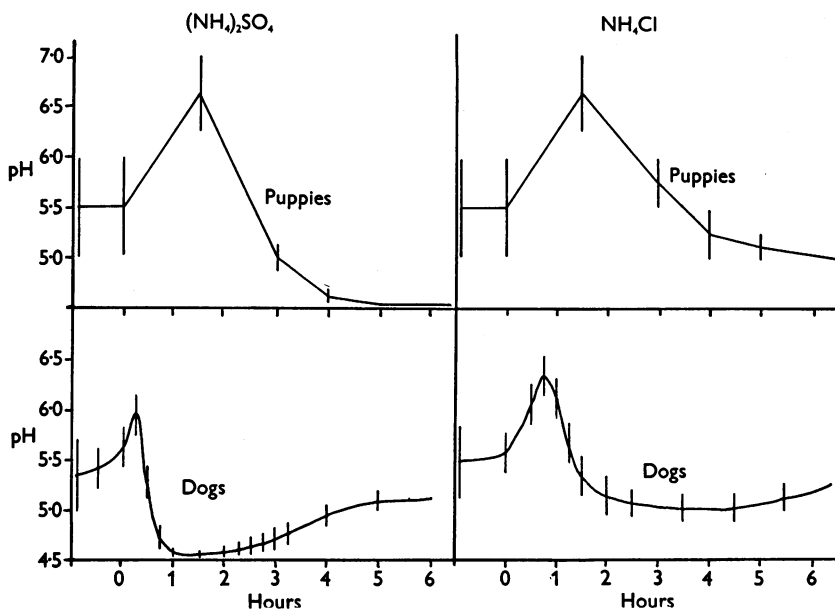


Fig. 3. Changes in the pH of the urine after the administration of the acidifying salts and water to dogs and puppies. Vertical lines indicate standard deviations.

of urine (see above). The subsequent fall in pH was greater and more rapid after $(\text{NH}_4)_2\text{SO}_4$ than after NH_4Cl , but the level ultimately attained was much the same whatever the age. In the puppies, however, the pH of the urine was

reduced more slowly than in the dogs and it then remained low until the end of the experiment, whereas by this time the pH of the dogs' urine had recovered to a level not much below the initial value.

The excretion of phosphates and other buffer substances in the urine

Fig. 4 shows the excretion of P after the administration of the two salts. It will be seen that: (a) $(\text{NH}_4)_2\text{SO}_4$ produced a somewhat greater response at both ages; (b) in young puppies the rate of excretion of P/kg of body weight was initially very much smaller than in dogs, and did not increase to the same extent after the acidifying salts. Furthermore, the time at which the increase took place suggests that it may have been due to the diuresis rather than the acidosis. Since the animals all received doses which were equal in terms of body weight, the P buffers in puppies' urines could only have covered the excretion of a relatively small fraction of the acid administered. The increased excretion of creatinine may have compensated for this to a trifling extent in these experiments but might not have done so in the absence of a diuresis.

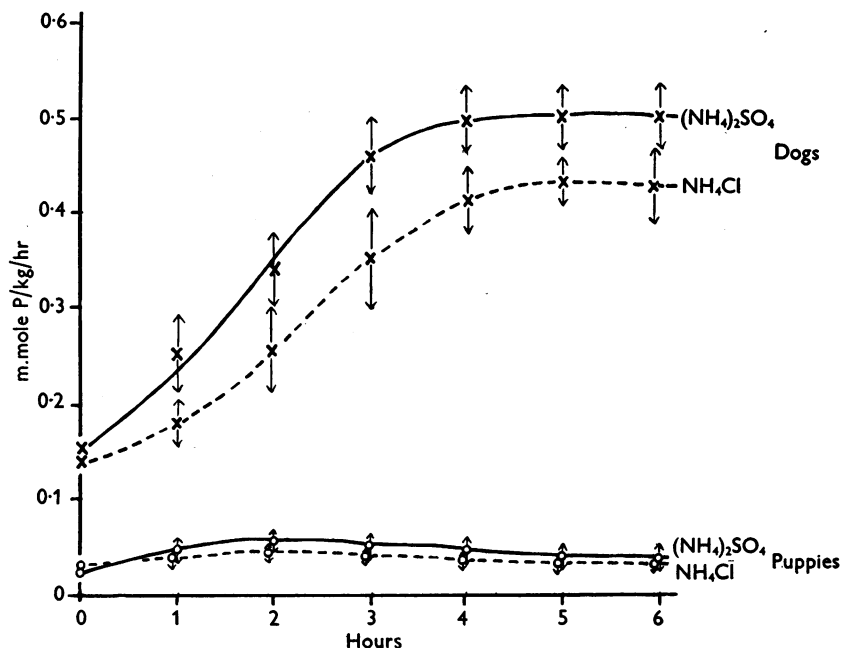


Fig. 4. The excretion of inorganic P after the administration of water and acidifying salts. The vertical arrows indicate standard deviations.

The rate of excretion of P increased rapidly with age, and the response to the acidosis had assumed the adult form by the second week but was still quantitatively inferior to it at that time.

appear to affect the present experiments for they were of short duration in both dogs and puppies and some time was allowed to elapse between any two experiments on the same dog, but Table 2 shows that the present results could not have been due to the ammonia output of the dogs having been raised by 'training'. NH_4Cl (9.9 m.equiv (3.3×3) /kg of body weight) were given daily, and the output of ammonia was measured for 2 hr after each administration. The rate of production after this relatively small acidifying dose was no higher on the third day than it had been on the first.

The excretion of sodium and potassium

Fig. 6 shows the average rates of excretion of K and of Na after the administration of $(\text{NH}_4)_2\text{SO}_4$. There are similarities between the puppies and the dogs, and at the same time differences. At both ages there was an increased excretion *first* of K and *then* of Na after the water and the acidifying salt had been given. Some increased excretion of fixed base had been anticipated from previous work, but the prior excretion of K followed by that of Na was not expected and does not seem to have been described before. It is not easy

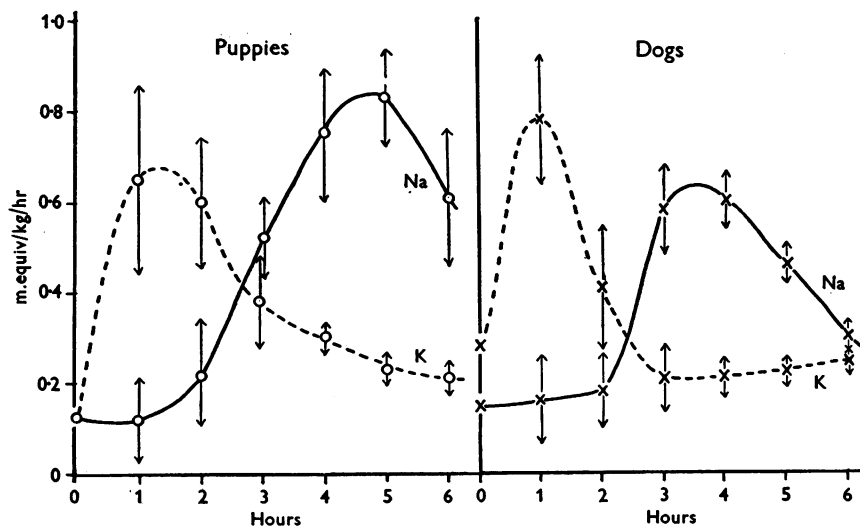


Fig. 6. The excretion of sodium and potassium after the administration of water and $(\text{NH}_4)_2\text{SO}_4$. The vertical arrows indicate standard deviations.

to explain it at present, but it will be noted that the two waves in the puppies had exactly the same time relationship as they had in the dogs. It is possible that the wave of K excretion was brought about by the diuresis and that of Na by the acidosis (McCance & Widdowson, unpublished). There were several differences between the puppies and dogs. The initial rate of excretion of K was considerably lower in the puppies. The peak rate of excretion of K was also

lower in the puppies but that of Na higher. The increase in the rate of excretion of both K and Na above the basal level was greater in the puppies.

The excretion of Na and K responded to the administration of NH_4Cl in a similar way. The time relationships were the same and the peak rate for K again preceded that for Na. The increases in the rates of excretion of K and Na above the basal level were somewhat smaller both in dogs and puppies than the increase after $(\text{NH}_4)_2\text{SO}_4$.

The position within the body after 6 hr

Table 3 shows the total amount of H ion excreted by the dogs and puppies within 6 hr of the administration of the salts. It also shows the superfluous anions eliminated as ammonium salts and the anions excreted in combination with Na or K. The figures have been arrived at after allowing for the rates of excretion exhibited during the preliminary control period, and all are expressed as a percentage of the dose given. The excretion of H ions was trifling in the puppies because the ions could not be excreted in the 'free' state owing to the paucity of phosphate buffers in the urine, nor could they be excreted after neutralization because the mechanism for effecting this by ammonia was so undeveloped. In contrast with this the dogs excreted acid equivalent to 61.2% of the dose within 6 hr after being given NH_4Cl and to 79.8% after being given $(\text{NH}_4)_2\text{SO}_4$, most of it in each case neutralized by ammonia.

TABLE 3. The H ions and the superfluous anions excreted by puppies and dogs within 6 hr of the administration of 3.3 m.equiv of NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$ /kg of body weight. Averages and the standard errors of the mean are expressed as a percentage of the dose administered

| Drug | Age | H ions | | | |
|------------------------------|--------|----------------------------|------------------------|-------------|-------------|
| | | With PO_4 buffers | As NH_4 salts | Total | |
| NH_4Cl | 2 days | 0.88 ± 0.12 | 0.43 ± 0.07 | 1.31 ± 0.19 | |
| | Adult | 12.5 ± 1.3 | 48.7 ± 3.4 | 61.2 ± 4.2 | |
| $(\text{NH}_4)_2\text{SO}_4$ | 2 days | 0.22 ± 0.03 | 0.54 ± 0.04 | 0.76 ± 0.08 | |
| | Adult | 17.5 ± 1.8 | 62.3 ± 4.3 | 79.8 ± 5.2 | |
| | | Anions | | | |
| | | As NH_4 salts | As Na salts | As K salts | Total |
| NH_4Cl | 2 days | 0.43 ± 0.07 | 31.9 ± 2.8 | 17.5 ± 1.7 | 49.8 ± 3.5 |
| | Adult | 48.7 ± 3.4 | 26.2 ± 2.1 | 8.1 ± 0.6 | 83.0 ± 5.6 |
| $(\text{NH}_4)_2\text{SO}_4$ | 2 days | 0.54 ± 0.04 | 45.1 ± 3.2 | 24.1 ± 1.9 | 69.7 ± 4.7 |
| | Adult | 62.3 ± 4.3 | 48.0 ± 3.3 | 5.4 ± 1.0 | 115.7 ± 7.9 |

Owing to their poor NH_4 production the puppies were able to excrete practically no anions as ammonium salts, whereas the dogs excreted 48.7% of the dose in this form after NH_4Cl and 62.3% of it after $(\text{NH}_4)_2\text{SO}_4$. Six hours after being given NH_4Cl the puppies had only excreted *total* anions equivalent to 49.8% of the dose and to 69.7% of the dose after $(\text{NH}_4)_2\text{SO}_4$; 17.5 and 24.1% respectively were excreted as K salts and the remainder mainly as Na salts. In all, 49.4 and 69.2% of the superfluous anions were excreted as Na or K

salts after the administration respectively of NH_4Cl and $(\text{NH}_4)_2\text{SO}_4$. This process involved an equally large loss of fixed base and did nothing to correct the acidosis. The dogs, however, excreted total anions equivalent to 83% of the dose after the NH_4Cl and to 115.7% after the $(\text{NH}_4)_2\text{SO}_4$. They excreted much less of this as K salts than the puppies, and the superfluous anions excreted with fixed base only amounted to 34.3 and 53.4% of the dose.

It may be reckoned, therefore, that the puppies still contained nearly all the H ions and equivalent anions in their bodies 6 hr after the dose had been given, whereas the dogs contained only 27–38% of the H ions and 46–50% of the anions. Furthermore, the puppies had lost more fixed base/kg of body weight and were, therefore, in a correspondingly worse position.

DISCUSSION

The results are clear enough in themselves and require little discussion. The response of the puppies to an acidosis cannot be compared with the way in which the human infant or other animals react, for the new-born of other species have not yet been studied beside adult controls with the same completeness. It would appear from the work of Gordon *et al.* (1948), however, that the human infant is relatively more mature.

The small amount of phosphate excreted by puppies 2 days old seems to be the counterpart in this species of the low phosphate clearances which have been recorded in human infants. Dean & McCance (1948) pointed out that the small amount of phosphate being excreted by breast-fed infants must handicap them in correcting an acidosis, and the present work supports this suggestion.

The present work shows that the body's response to a sulphate acidosis differs from one produced by the Cl ion. The results suggest that this is because the sulphate ion, which is normally an end product of metabolism, is more rapidly excreted than the more physiological Cl ion, even when each is present in equal excess. It must be remembered in this connexion that an 'equal excess' produces a much larger departure from the normal concentration of SO_4 ions than of Cl ions in the extracellular fluids. At all events both in dogs and puppies the sulphate acidosis led to a greater excretion of fixed base and the dogs excreted more ammonia and phosphate. It was in fact a more searching test of renal function.

The present experiments were not designed to find out at what age puppies acquire the same capacity as adults to correct an acidosis, but some of the puppies were tested when they were 1 day old, and again when they were 14 days old. The results, particularly those for phosphate and ammonia, demonstrated that there was a somewhat better response at 2 days than at 1 day, but that by the end of the second week the response was still not quite so good as it was in adult dogs. This indicates that functional maturity in

correcting an acidosis was likely to have been reached at a relatively early age, certainly before the puppies were weaned, and that therefore it was the result of age and not of diet.

SUMMARY

1. When puppies (2 days old) and adult dogs were given comparable doses of water and ammonium chloride or sulphate, the puppies: (i) excreted the water less rapidly and completely than the dogs; (ii) excreted a urine with as low a pH; but (iii) excreted much less free acid in the urine owing to the small amount of phosphate buffers in it; (iv) excreted practically no ammonia; (v) excreted less of the superfluous anion and most of that in association with fixed base.

Puppies therefore showed themselves functionally immature in the way in which their kidneys responded to an acidosis.

2. The administration of the acidifying salts and water to both dogs and puppies led to the appearance of a wave of K ions in the urine followed by a wave of Na ions.

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