EXPERIMENTS ON THE REGULATION OF THE BLOOD'S ALKALINITY. II.

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DAVIES, KENNAWAY, and the author(1) showed that in the alkalosis produced in man by the ingestion of large amounts of $NaHCO_3$ the alveolar CO_2 and the blood's CO_2 capacity rise, the urine becomes alkaline, and the ammonia excretion falls or ceases. Attempts were therefore made to produce an acidosis and study the corresponding symptoms. Up to 500 c.c. of ·116 normal HCl were drunk within 80 minutes without producing any definite fall in the alveolar CO_2, though the acid and ammonia excreted both per litre and per hour increased distinctly. Again 30 grams of $NaH_2PO_4.H_2O$ were taken without producing any fall in the alveolar CO_2, or rise in the ammonia excretion, though the titratable acid of the urine rose to ·114N. This agrees with the results of Marriott and Howland(2). Larger quantities of HCl and phosphate were not taken, as the former caused slight gastric discomfort, the latter diarrhœa.

I was led to try the effect of ingesting NH_4Cl by the observation of Palmer and Henderson(3) that the ratio of excreted ammonia to acid (titrated to phenolphthalein) remained fairly steady under various conditions. It seemed probable that this might be due to equilibrium being reached in a reversible formation of urea and acid from ammonium salts. I therefore took amounts of NH_4Cl varying from 5 to 55 grams on six different occasions. Two of these experiments lasted for a week each.

The alveolar CO_2 pressure was measured by the method of Haldane and Priestley(4), the sample being taken directly into the burette of the gas analysis apparatus from the rubber tube. Each recorded value was the mean of two samples taken at the end of inspiration and of expiration. Thus during a normal day the mean of ten consecutive pairs of half-hourly observations was 38·25 mm. The extreme values were 39·4 and 37·3 mm. and the probable error ·23 mm. or 0·6 p.c. The actual errors were however undoubtedly less than this, since the general trend of the observations showed that the true value varied slightly

during the period. Such steady results can only be obtained if the samples are taken at least 2½ hours after a meal, since digestive secretion and absorption disturb the alveolar CO_2. It is also essential that the subject should be trained to breathe regularly. Blood CO_2 capacities were determined on defibrinated blood by J. S. Haldane's(5) latest method, which is accurate within about 0·5 p.c.

The acid and ammonium salts of the urine were estimated by Malfatti's(6) method of titration with N/10 NaOH to a standard colour with phenolphthalein before and after the addition of neutralized formaldehyde sclution. This method is liable to the criticisms that it overestimates the acidity and includes amino-acids with ammonia. It was however adopted for the following reasons apart from its rapidity and simplicity. The ratio of acid to ammonia in the urine only remains approximately constant if the end-point of the acid titration is at a p_9H of about 9. For example a urine may contain ammonia but be alkaline to litmus or neutral red, so that if we titrated to either of these indicators the ratio would become negative. But as Davies, Haldane and Kennaway(1) showed, ammonia excretion ceases when the blood is so alkaline that large amounts (generally about ·3N) of bicarbonate are being excreted, in which case the urine gives a faint colour with phenolphthalein. Again the amino-acids may properly be included with the ammonia on the ground that in an acid urine they serve the same physiological purpose as ammonia in neutralizing acid, and must therefore be included if we wish to determine the total amount of acid excreted free and combined with non-metallic bases.

Urea was estimated by Marshall's(7) original direct titration method. The results are about 1·5 p.c. too low, but duplicates were found to agree within 0·10 p.c. Tap water may be used in place of distilled water if allowance is made for it in a blank experiment. Phosphates were estimated with uranium acetate and cochineal.

The NH_4Cl was in every case drunk dissolved in 40 times its weight of water, as any less dilution caused vomiting. This is largely a specific effect of the NH_4 ion, since 10 p.c. NaCl solution is tolerated.

The effect of 20 grams of NH_4Cl on the alveolar CO_2 is shown below. The alveolar CO_2 values in mm. Hg. taken at consecutive intervals of half-an-hour beginning at 12 noon were:

40·3	40·2*	38·9	38·0	37·8	37·6†	36·2	35·0†
35·1	34·4	34·2	34·3	34·3	34·0	34·0	33·7
(dinner)	36·2	34·8	34·9	34·4			

* Ten minutes later 10 grams NH_4Cl taken.
† Ten minutes later 5 grams taken.

On the next day the following values were obtained:

10.30 a.m.	12 noon	10 p.m.
33·2	34·5	35·9

On the third day the values were low, but within the normal range of variation.

It will be seen that the alveolar CO_2 fell fairly steadily for seven hours. The sharp rise to 36·2 after dinner was due to withdrawal of HCl from the blood for gastric digestion. The values on the following day were so low as to make it clear that the formation of acid was proceeding during the night. Hourly urines were collected on the first day of the experiment. They showed a great rise in the acid and ammonia excretions per hour, but as the volume per hour was increased fourteenfold the concentrations of both fell at first, and the ratios of acid, ammonia, and urea clearly depended as much on the water output as on anything else. It was therefore determined to study the excretions over longer periods where the volume effects would be less noticeable.

The urines were collected from midnight to noon and noon to midnight. Twenty grams of NH_4Cl were taken during the afternoons of days 2 and 3; 15 grams on the afternoon of day 4. Four meals of about the same size were taken on each day. Alveolar CO_2 samples, 80 in all, were taken at intervals, but only the averages of values at least two hours after meals are given. The results are recorded in Table I.

TABLE I.

Period	NH_4Cl taken	Alv. CO_2 in mm. Hg.	Urine in c.c.	Acid	NH_3	Acid + NH_3	Urea	Phosphate	$\frac{\text{Acid}}{NH_3}$	$\frac{\text{Ammonia N}}{\text{Am.} + \text{urea N}}$
1 A	—	38·8	1310	30·9	40·7	71·6	286	18·1	·76	·066
2 M	—	—	660	30·6	34·3	64·9	187	22·8	·89	·084
2 A	374	36·0	2040	26·5	42·6	69·1	310	15·6	·62	·064
3 M	—	—	1050	49·4	65·0	114·4	245	29·8	·77	·145
3 A	374	30·4	2840	61·6	92·9	154·5	435	25·5	·66	·096
4 M	—	—	860	58·1	95·8	143·9	231	40·7	·61	·172
4 A	281	27·8	2240	61·6	118·0	179·6	389	33·8	·52	·132
5 M	—	—	680	51·1	114·2	165·3	204	32·2	·45	·219
5 A	—	30·3	772	35·4	113·7	159·1	218	16·3	·31	·201
6 M	—	—	482	32·9	97·8	130·7	143	22·6	·35	·255
6 A	—	34·3	906	30·0	99·9	129·9	271	13·7	·30	·156
7 M	—	—	472	31·9	75·3	107·2	230	21·2	·42	·141
7 A	—	36·7	973	27·6	84·7	112·3	314	10·4	·33	·116
8 M	—	—	774	30·6	50·0	80·6	218	20·9	·61	·103
8 A	—	38·7	1310	26·7	56·5	83·2	278	7·6	·47	·092

The quantities ingested and excreted are given in c.c. of normal (in the case of phosphates, molar) solutions, *i.e.* milligram-molecules.

Period 3 M means the morning of the third day, and so on. It will be seen that as the result of the ammonium chloride ingestion the acid excretion per day was approximately doubled, the ammonia excretion trebled, whilst the alveolar CO_2 was reduced by 28 p.c. The lowest

alveolar CO_2's actually observed were 26·6 mm. on one occasion and 26·7 on two others.

Three samples of blood were drawn during the course of the acidosis, and two on normal days, and their CO_2 capacities determined. The results are given in Table II. In each case the blood was drawn about

TABLE II.

Time	CO_2 pressure mm.	c.c. CO_2 per 100 c.c. blood	c.c. CO_2 per 100 c.c. blood at 40 mm. pres.	CO_2 capacity as p.c. of normal	Alv. CO_2 pres. when blood drawn mm.
Normal	38·7	47·3	48·1	100	39·1
,,	36·3	46·7			38·4
3 A	41·4	33·2	32·8	68·2	31·1
4 A	36·2	27·2	28·2	58·4	27·4
6 A	43·5	44·2	42·8	89·0	33·5

4 p.m. The actual volume taken up at the pressure in col. 2 is given in col. 3. In col. 4 the volume taken up at 40 mm. pressure is calculated on the assumption that the CO_2 dissociation curve is unaltered except for the scale of its ordinates. This should be the case on Parsons' theory. The alveolar CO_2 increases with the blood's CO_2 capacity, but the two are not proportional.

When the alveolar CO_2 fell, there was very marked air-hunger. The increased frequency and depth of breathing were obvious. Thus at a time when the alveolar CO_2 was 29·7 mm. the volume breathed per minute when sitting in a chair was 10·4 litres, the normal value being about 6. Walking at three miles per hour caused severe panting, and cycling was impossible. There were occasional slight headaches. A certain exhilaration and irritability of temper were noticed at times by myself and others, but there was no mental confusion, and the experiment was not unpleasant. No increased bronchial secretion was noticed, although doses of less than 1 gram of NH_4Cl are prescribed for this purpose.

Urines examined both at the height of the acidosis and at the end of the experiment showed no albumin or sugar. There was no sign of a maximal value for the concentration of ammonia or titratable acid being reached. The highest concentration of acid recorded was ·075N, of ammonia ·193N, or ·33 p.c. The ammonia was not all excreted as chloride. Thus when the ammonia was ·193N the chloride was only ·118N. The earlier experiments seem to show that the chlorine ingested was excreted more rapidly than the ammonium.

The amount of phosphate excreted per day rose during the ingestion, since the acid excreted uncombined with ammonia is largely mono-

basic phosphate. On the other hand the phosphate excretion fell far below normal during the latter part of the experiment, even while some acidosis was still present, suggesting that a shortage of phosphate had been produced in the body during the height of the acidosis. Along with this the acid excretion fell to normal or slightly below normal, the ratio of acid to ammonia excreted being very low. That this was due to deficiency of phosphate is shown in Table III. The amounts of NH_4Cl

TABLE III.

Period	NH_4Cl taken	Phosphate taken	Alv. CO_2	Volume	Acid	NH_3	Acid + NH_3	Phosphate	$\frac{\text{Acid}}{NH_3}$
0 A	—	—	40·3	1595	28·1	33·7	61·8	21·7	·83
1 M	—	—	—	940	31·0	35·6	66·6	25·9	·87
1 A	—	—	39·3	1565	27·1	36·8	63·9	17·5	·74
2 M	—	—	—	792	30·2	32·6	62·9	23·1	·92
2 A	374	—	36·6	2005	48·5	63·2	111·7	24·1	·77
3 M	—	—	—	1325	54·3	88·4	142·7	33·8	·61
3 A	374	—	32·1	2690	54·9	100·9	155·8	28·5	·54
4 M	—	—	—	978	54·6	105·5	160·1	34·8	·52
4 A, 12–6	281	—	30·0	528	23·2	57·8	81·0	14·0	·40
4 A, 6–12	—	250	30·5	480	51·3	51·6	102·9	39·8	·99
5 M	—	—	—	765	77·3	121·4	198·7	69·2	·64
5 A	—	125	35·4	880	62·7	114·4	177·1	48·8	·55
6 M	—	—	—	715	55·1	61·2	116·3	53·2	·90
6 A	—	125	37·7	1342	42·1	65·5	107·6	40·8	·64
7 M	—	—	—	677	45·8	36·5	82·3	55·0	1·25
7 A	—	—	40·1	—	—	—	—	—	—

taken were the same as in Table II, but phosphate was taken in addition. A litre of M/2 sodium phosphate solution was made up containing 27·6 grams, or 0·2 gram molecule of $NaH_2PO_4 . H_2O$, and 107·4 grams, or 0·3 gram molecule of $Na_2HPO_4 . 12H_2O$. This solution is feebly acid, its *p*.H being about 6·5. Half a litre was drunk between 5.30 and 9 p.m. on day 3, and 250 c.c. each on the evenings of days 4 and 5. There was slight diarrhœa, so much of this phosphate never reached the blood. Two normal days were recorded beforehand, the first being numbered 0, so that corresponding days in Tables I and III bear the same numbers. The fourth afternoon sample was divided into two portions, before and after 6.17 p.m., to show the influence of the phosphate ingestion. The alveolar CO_2's are not so accurate as those of Table I, being the averages of only 52 samples. It will be seen that on this occasion the acid excretion rose at once with the ingestion of phosphates while the ammonia excretion and the alveolar CO_2 returned to normal much more rapidly than in Table II, and the acid : NH_3 ratio never fell below ·5.

DISCUSSION.

It is clear that NH_4Cl ingestion produces a true acidosis, since the alveolar CO_2 and blood CO_2 capacity fall, and the acid excretion rises.

One of these criteria alone is not sufficient. For example pain lowers the alveolar CO_2, forced breathing lowers the blood CO_2 capacity, and acid phosphate ingestion raises the acid excretion, but none of these causes acidosis. The results obtained are exactly parallel to those of Salkowski(8) who found that NH_4Cl injected into dogs was only excreted as such to the extent of about two-thirds. In rabbits on the other hand very little was excreted unchanged, and the urine became acid. In my own case (Table II) about 13 p.c. of the NH_4Cl ingested was excreted as acid, and the ammonia must therefore have been transformed into a neutral body, and ultimately excreted in a neutral form, presumably as urea. It is quite possible that its immediate effect was to shield amino-acids from de-amination in the liver and elsewhere, as evidenced by its protein-sparing effect in starvation. This however cannot go on indefinitely, and ultimately some such reaction as:

$$2NH_4Cl + CO_2 \rightleftharpoons CON_2H_4 + H_2O + 2HCl$$

must occur. My experiments furnish no direct evidence of urea formation from ammonium chloride. In the last six days of the experiment, Table III, ·128 gram-molecule of acid, ·600 gram-molecule of NH_3, and ·470 gram-molecule of urea in excess of the normal were excreted. The diet was only roughly constant, and much of the urea excretion was obviously due to the large volumes of urine produced. The increase of ·064 gram-molecule to be expected to correspond to the extra acid was entirely masked by this cause.

The reaction postulated above is represented as reversible. Three lines of argument go to suggest that this is the case. In the first place the reaction is incomplete, in fact most of the ammonium is excreted unchanged. Now such an equilibrium is characteristic of reversible reactions only. Secondly the results of hydrogen chloride and ammonium chloride ingestions are apparently identical. Begun, Hermann and Munzer(9) found that during nine days of HCl ingestion, the average amount taken being 67·7 c.c. N acid per day, the average acid excretion was increased from 45·9 to 66·5 c.c. N per day, the ammonia from 42·2 to 80·6 c.c. N. That is to say about two-thirds of the total ingested was excreted as ammonium salt. In my own case about two-thirds was excreted in this form in the earlier part of the experiment, Table II, and although in the later part no extra acid was eliminated, the results in Table III show that this was wholly due to lack of phosphate. As however the acid ingested by Hermann per day was only equivalent to one-fifth of my average ammonium chloride ingestion, it is natural

that he never ran short of phosphate. Indeed his phosphate excretion only rose very slightly during the acid ingestion. Since, then, the reaction seems to proceed to the same equilibrium point whether we ingest hydrogen or ammonium chloride, it must be reversible. Finally, Steenbock, Nelson and Hart(10) showed that the extra ammonia excreted by calves after acid ingestion is produced entirely at the expense of urea.

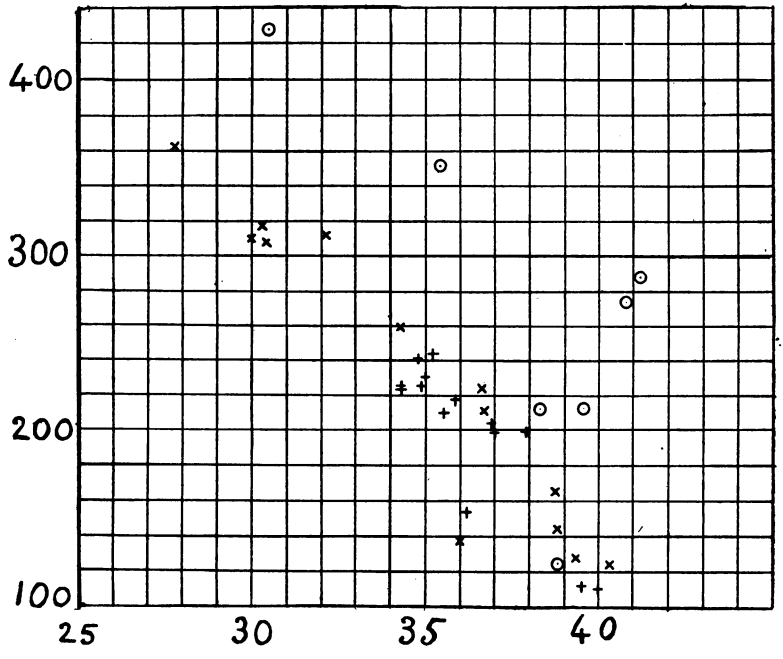

Fig. 1.

Abscissa. Alveolar CO_2 pressure in mm. Hg.
Ordinate. Millimoles acid + NH_3 excreted per day.

× Twelve and six hour urine samples.
\+ One hour urine samples.
⊙ Urine samples after phosphate ingestion.

The rates of excretion of acid alone or of ammonia alone do not depend on the degree of acidosis as shown by the alveolar CO_2, but the sum of these rates, which gives the rate at which free and neutralized acid together are excreted, seems to depend mainly on the alveolar CO_2, and very little on the rate at which water is being excreted. In Fig. 1 the rates at which acid and ammonia were excreted during 14 periods of one hour, one of six hours, and 12 of twelve hours, are plotted against

the mean alveolar CO_2 pressures during the corresponding periods. With two exceptions the points so obtained lie near a continuous curve. They can be approximately fitted by the straight line $y = 20\,(46{\cdot}5 - x)$ where x is the alveolar CO_2 pressure in mm. of mercury, y the rate of excretion of ammonia (including amino-acids) and titratable acid per day, in milligram-molecules. Whether the line is straight or not it is clear that the main factor determining the excretion rate is the alkalinity of the blood, as measured by the alveolar CO_2. Since the rate of excretion of urine varied between 44 and 497·5 c.c. per hour, or 1·06 and 11·94 litres per day, it is obvious that the rate of excretion of acid and ammonia is independent of that of water over a very wide range. I am inclined to think that during very slow secretion the rate falls off. For comparison I have added seven results recorded after drinking NaH_2PO_4 solution or the nearly neutral phosphate solution of Table III. Five of these lie a long way off the curve. Clearly superfluous dibasic phosphate ions are excreted independently of those which are required for the normal excretion of acid.

Fitz and Van Slyke(11) observed a quite different relation in diabetic acidosis. The divergence may probably be explained by the fact that the kidneys can excrete free organic acids, but not free HCl.

Although the rate of excretion of acid + ammonia varies with the under-alkalinity of the blood as shown by the alveolar CO_2, the ratio of acid to ammonia excreted depends mainly on the amount of phosphate available for excretion, though it is also probably a function of the urinary volume. When there is a lack of phosphate, as in the latter part of Table II, the acid excretion falls below normal, even during marked acidosis, and the acid : ammonia ratio may fall below one-third. When there is a plethora of phosphate as in the latter part of Table III, the ammonia excretion is relatively low, and the ratio may reach or exceed unity. It is probable therefore that very low values of this ratio, such as that of ·05 recorded by Gilliatt and Kennaway(12) in a case of pernicious vomiting of pregnancy, betoken a shortage of phosphate.

The ratio of ammonia nitrogen to total nitrogen or ammonia + urea nitrogen is often used as a criterion of acidosis. The rate of excretion of urea varies however both with the volume of the urine, as is very evident in Tables I and III, and with the amount of protein in the diet. In these experiments the ratio of ammonia nitrogen to ammonia + urea nitrogen only once exceeded 25 p.c., but a large amount of protein was being eaten, and had this not been so much higher values would doubtless have been recorded.

The absence of glycosuria, though plenty of carbohydrates were being eaten, is interesting in view of Elias'(13) observation that acid ingestion lowers the carbohydrate tolerance in dogs and rabbits. These animals had however received larger doses of acid per kilogram than the equivalents of acid ingested in my own case.

The sequence of events after ammonium chloride ingestion appears to be roughly as follows. The salt passes very rapidly into the blood, and presumably in the liver a proportion, probably a smaller part, is transformed into urea and acid which goes to lessen the alkaline reserve of the blood and other tissues. In the experiment given on p. 266 for example, the alveolar CO_2 pressure fell 1·3 mm. within 20 minutes of ingesting 10 grams of NH_4Cl. The determinations of the blood's CO_2 capacity during the acidosis, given in Table III, enable us to form a rough idea of the fate of the liberated acid. At the height of the acidosis the blood's CO_2 capacity per 100 c.c. at 40 mm. was reduced by 20 volumes. Now Davies, Haldane and Kennaway(1) showed that the blood's total combining capacity with CO_2 is about double its capacity at 40 mm. That is to say at 40 mm. pressure the alkali of the blood is about equally divided between carbonic acid and other buffers, of which hæmoglobin appears to be the most important. Hence a reduction of 20 volumes p.c. or ·009N in the CO_2 capacity at 40 mm. corresponds to a reduction of ·018N in the total alkali of the blood. Taking my blood volume at about 5 litres, the reduction of its alkali corresponds to about ·09 gram-molecule. At the time when this blood was drawn 1·03 gram-mol. of NH_4Cl had been ingested, and about ·26 gram-mol. excreted as acid or ammonia in excess of the normal. There was thus ·77 gram-mol. in the body. But only a fraction of this had been converted into acid. Had this acid been evenly distributed through the soft parts, taking their weight at 77 kgm., the decreased alkalinity would everywhere have been a fraction of ·01N, but that of the blood was decreased by ·018N, hence more than twice as much acid proportionately remained in the blood as in the other soft tissues on the average, and more than one-eighth of the whole acid remained in the blood. Had the acid been ingested directly the results would probably have been similar.

At the time when the above sample was taken the kidneys were excreting at the rate of ·36 gram-mol. of acid + ammonia per day against a normal rate of about ·13, *i.e.* ·23 gram-mol. per day extra. That is to say they were eliminating 30 p.c. of the total excess in the body per day. This contrasts unfavourably with the rate at which they can deal with excess of alkali, since in the first paper of this series it was

shown that one-seventh of the excess of alkali in the body could be eliminated in an hour, so that excess of base is eliminated 11 times as fast as excess of acid. The difference is no doubt largely due to the fact that bicarbonate can be concentrated to ·3N in the urine without increasing its p.H to more than 8·5, whilst ·3M acid phosphate solution has a p.H of less than 4, which appears to be beyond the power of the kidney to secrete.

It has been shown by L. J. Henderson(14), Hasselbalch(15), and others that the hydrogen ion concentration of plasma depends on the ratio of the concentrations of CO_2 and HCO_3. The change in the C.H. of the arterial plasma caused primarily by increasing or decreasing the CO_2 content we may call gaseous acidosis or alkalosis, that caused by decreasing or increasing the bicarbonate content non-gaseous acidosis or alkalosis. Haggard and Y. Henderson(16) showed that gaseous acidosis and alkalosis are gradually compensated by an alteration in the bicarbonate content which tends to restore the normal C.H., whilst Leathes(17), Davies, Haldane, and Kennaway(1), and others proved that this change is partly effected by the kidney.

On the other hand the lungs play the principal part in compensating the non-gaseous disturbances. The relative amounts of compensation effected by the lungs and kidneys when acid is being passed into the blood may be calculated as follows. Suppose the arterial plasma bicarbonate to be reduced by 25 p.c., then for complete compensation the alveolar CO_2 would have to be reduced in the same proportion, *i.e.* from 40 to 30 mm. This would increase the lung ventilation by about one-third. Now Campbell, Douglas, Haldane and Hobson(18) showed that an increase of about 1·5 mm. at 40 mm. in the alveolar CO_2 is required to double the normal lung ventilation. This amounts to 3·75 p.c. of the total. Hence in the case considered the alveolar CO_2 must actually be about 1·25 p.c. or ·375 mm. higher than it would otherwise be, *i.e.* 30·4 mm. instead of 30. Thus about 96 p.c. of the acidosis is compensated for by the respiratory centre, which however needs the remaining 4 p.c. as a stimulus to the increased ventilation.

I have to thank Dr J. S. Haldane for assistance with the blood-gas analyses and Dr E. F. Adolph for performing some of the phosphate estimations. Dr Adolph and Dr Kennaway have obtained similar depressions of their alveolar CO_2 from NH_4Cl ingestion, and kindly permit me to publish the fact.

SUMMARY.

1. Ingestion of ammonium chloride in man causes marked and prolonged acidosis. The symptoms include fall in the alveolar CO_2 and in the blood's CO_2 capacity, and increased excretion of acid, ammonia, and phosphates.

2. This is probably due to the conversion of part of the ammonia into urea, thus freeing the acid which had been combined with it.

3. The depression of the alveolar CO_2 below a certain value is roughly proportional to the rate of excretion of acid plus ammonia.

4. The ratio of acid to ammonia in the urine depends on the amount of phosphates available for excretion. This amount falls during acidosis.

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