A M M O N I A M E C H A N I S M.

An Experimental Study of the Part played by Ammonia, Fixed Base and Phosphate in the Elimination of Acid from the Body.

T H E S I S

for the Degree of Doctor of Medicine, Edinburgh University. 1925

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Fellow Rockefeller Foundation.

13th Feb. 1925.

Certified that the entire experimental work was done and the Thesis composed by me.

Major, I.M.S.

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P A R T I.

LITERATURE.

(1) Ammonia and Fixed Base Excretion.

Gaethgens (1872) was practically the first to make quantitative determinations of base excretion after mineral acid administration to an animal. He experimented with a dog weighing 25·8 kgms., which was kept on a constant diet of fresh meat right through the experiment. After a preliminary period of four days he gave, by mouth, dilute sulphuric acid for a period of seven days.

His figures converted into terms of decinormal solutions are[1]:-

	c.c. of 0·1N solution.					
Average per day	Na	K	Ca	Mg	Total base	H_2SO_4
Fore-period	312	386	46	90	834	684
Acid period	399	331	145	125	1000	1785

1. The various works quoted give results in a variety of different ways. For the sake of uniformity and convenience of reference and comparison with results obtained by the present author, all the results have been recalculated and expressed in terms of decinormal solutions in this paper.

While there is an increase of 1101 c.c. of inorganic sulphate in twenty-four hours, there is an increase of only 166 c.c. of base per day over the normal fore-period. The huge increase in Ca excretion is to be noted.

The author, unfortunately, does not give the quantity of acid administered and gives no figures for after-period; it is not possible to interpret the results further. But it is interesting to note his conclusion that since the amount of base passed out in urine was not enough to neutralise all the acid excreted, the excess of acid over the base must represent, he held, the excretion of free acid in urine. It is now known that kidney can not excrete free mineral acids. But he supported the point of view generally held at the time, that kidney could excrete free mineral acids, and that the administration of acids did not deprive the animal body of its base.

This point of view was first put forward by Von Eylandt (1854) in a dissertation "De Acidorum Suptorum Vi in Urinæ Acorem". He gave results of some experiments performed on human subjects. Dilute mineral and vegetable acids were given by the mouth and the acidity of urine titrated before and after acid administration. In every case he found titratable acidity of urine increased, so much so that as much alkali was required to neutralise the increased

acidity as the acid given. Next year(1855) Von Wilde in "Disquisitiones Quaedem de Alcalibus per Urinam Excretis" reported base determinations of urine of human subjects before and after acid administration. He reported that there was no increase in base excretion, and came to the same conclusion as Eylandt, that it was impossible to extract alkalis from the body. Bence Jones (1849) had also made the observation that the exhibition of tartaric acid increased the acidity of urine. These observations are of historical interest only, as the methods of analysis were not such as would justify our making any use of their work.

Salkowski (1873) held that acids after absorption combined with the alkali of blood and absorption took place to such an extent as they could combine with this alkali. The alkalinity of blood was hardly affected in the process, he explained, because the absorption took place slowly and the kidney kept excreting the absorbed acid as fast as it was absorbed.

He believed the sulphur of food protein was first oxidised to thiosulphuric acid, and its further oxidation to H_2SO_4 did not take place until it got into the kidney. And the kidney passed it in the urine free. To substantiate this hypothesis he exhibited taurine $\begin{matrix} CH_2 \cdot NH_2 \\ | \\ CH_2 \quad SO_3H. \end{matrix}$ to rabbits and expected increased excretion of H_2SO_4 in urine. He did find this increase, but he also found marked increase in the base excreted.

One of his experiments, which gives figures both for fore-period and acid-period, is quoted below to illustrate this.

Rabbit 1200 gms. weight.

Fore-period 48 hrs. - animal starved.

Acid (H_2SO_4) 29 c.c. 0·1 N) excreted in
) urine in 48
Total base 70 c.c. 0·1 N) hrs.
(less chloride)[1].

Acid period 48 hrs. - during which two doses of 2 gms. each of Taurine were given, the animal was starved during this period also, but between this period and the preliminary fore-period a day of normal wheat feeding had intervened.

Acid (H_2SO_4) 119 c.c. 0·1 N

Total base (less chloride)[1]. 129 c.c. 0·1 N.

Thus there is an increase of 90 c.c. 0·1 N acid after taurine, equivalent to 644 c.c. of 0·1 N acid. There is an increased excretion of 70 c.c. 0·1 N fixed base during the same period. Therefore 78% of the increased acid was excreted combined with base.

This experiment was at variance with the experiment of Gaethgens quoted above, and the opinion generally held at the time. It showed definite and marked excretion of base from the body after acid administration. Salkowski

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1. The reason for the subtraction of chloride will appear in the body of the paper later.

explained this discrepancy by the fact that he had employed a herbivorous animal, while others before him had used dogs or experimented on human subjects. By actual ash analysis of tissues he showed that the ratio of base to acid in the tissues of herbivora was much greater than in that of carnivora. He attributed this to the large quantity of base in the food of herbivora. And on account of the large quantities of easily separable base present in the tissues, it was employed to neutralise acid.

Walter (1877) while conducting experiments to determine the nature of acid intoxication in rabbits and dogs observed that the total CO_2 content of blood fell very markedly in rabbits after the administration of 250 c.c. 0·1 N HCl per kilogram weight - i.e. from 40 vol. per cent to 3 vol. per cent, While the administration of a similar dose to dogs did not have a noticeable effect on the CO_2 content of blood. He surmised that in the case of carnivora the body must have a mechanism other than the alkali of the blood for the neutralisation of administered acid; it must be producing a base from its own material. Such a base, he thought, could only be Ammonia. He set out to determine this by experiment. He employed Schmiedberg's method of Ammonia determination and obtained the following

results (all the results are given converted into terms of $N/_{10}$ solutions).

Weight of dog - 11 kgm.

Daily food 500 gms. horse flesh.

Days.	HCl given c.c.0·1 N	NH_3 in Urine c.c.0·1 N.	
1	0	387	daily average 338.
2	0	276	
3	0	310	
4	0	378	
5	1000	710	
6	1000	860	
7	660	790	
8	330	800	
9	0	690	
10	0	298	

Total Acid given - 2990 c.c. 0·1 N

Excess of Ammonia 2180 c.c. 0·1 N.,

i.e. 73% of the acid given.

Result expressed by days:-

			Increase - NH_3 0·1 N	
1st day -	1000 c.c. acid,	i.e. 91 c.c. per kg.	= 372 c.c.	37%
2nd day -	do.		= 522 c.c.	52%
3rd day -	660	i.e. 60 c.c. per kg.	= 452 c.c.	68%
4th day -	330	i.e. 30 c.c. " "	= 462 c.c.	140%

Walter did not observe any increase in Ammonia excretion after administration of acid to rabbits.

Gaethgens (1880) made determinations of both ammonia and fixed base in urine of dogs after administration of mineral acid. His figures converted into terms of decinormal solutions are given below:-

L. Dog, wt. 20 kg., Food: 300 gm. extracted flesh per day.

Periods.	Day of Expt.	c.c.	c.c. 0·1N								c.c. 0·1M	c.c. 0·1N.
		Vol.	Na	K	Ca	Mg	Sum	Sum -Cl	NH_3	H_2SO_4	H_3PO_4	Cl.
Fore-period	7	700	-	-	14	18			600	540	340	96
	8	693	80	183	11	17	291	161	740	620	430	130
Acid period 1430 c.c. 0·1N H_2SO_4	9	650	168	272	13	16	386	386	1210	1570	380	83
After-period	10	760	62	197	19	18	185	185	1000	830	340	111
	11	715	51	205	14	21	163	163	960	650	290	128
						Excess		250	950	1190		

II. Dog., wt. 20 kg., Food: 400 gm. fresh meat plus 50 gm. fat.

Periods	Day of Expt.	c.c.	c.c. 0·1N								c.c. 0·1M.	c.c. 0·1N.
		Vol.	Na	K	Ca	Mg	Sum	Sum -Cl	NH_3	H_2SO_4	H_3PO_4	Cl
Fore-period	4	860							258	120	232	84
	5	870			8	25			348	108	234	102
	6	860	86	329	5	23	443	369	259	121	246	74
820 c.c. 0·1 H_2SO_4	7	830	72	365	14	33	484	442	457	590	236	42
1430 c.c. 0·1 H_2SO_4	8	790	98	393	22	32	545	494	750	1015	268	51
1020 c.c. 0·1 H_2SO_4	9	880	65	297	21	19	402	307	925	900	280	95
After-period	10	945	20	145	15	12	192	2	850	243	210	190
	11	785	34	163	12	18	227	72	236	151	202	155
	12	865			9	15				96	216	20
					Excess			190	1950	2290		

Summary of the table:-

1st Dog. Weight:20 kgs. Dose per kilo 71·5 c.c.
Acid given 1430 c.c. 0·1 N H_2SO_4
Increase in NH_3 1st day - 540 c.c. 0·1 N, i.e. 37·5% of the acid given.
Increase in NH_3 for the whole expt. - 950 c.c. 0·1N, i.e. 80%.

2nd Dog. Weight: 20 kgs.
1st day 820 c.c. 0·1 N H_2SO_4, i.e. 41 c.c. per kg.
Increase in NH_3 169 c.c. 0·1 N., i.e. 21%

2nd day 1430 c.c. 0·1 N H_2SO_4, i.e. 71·5 c.c. per kg.
Increase in NH_3 462 c.c. 0·1 N., i.e. 32%

3rd day 1020 c.c. 0·1 N H_2SO_4, i.e. 51 c.c. per kg.
Increase in NH_3 637 c.c. 0·1 N., i.e. 62%.

It is to be noted that there are probably serious analytical errors in this work as reported. In Experiment I the sum of the bases is sometimes less than the sum of the acids determined. The figures for H_2SO_4 in Experiment II are evidently much too low, if judged by the ratio of N to H_2SO_4 in fasting animals, especially cats, given below.

Coranda (1879) gives the results of an experiment of Hallervorden's on a human subject. The subject was kept on a constant mixed diet of flesh, milk and beer and bread. After a fore-period of five days, he gave dilute hydrochloric acid for two days and watched the subject for three days afterwards, but stopped the experiment before NH_3 excretion had returned to normal. He does not give any figures for fixed base excreted.

His figures are:-

Days.	Acid given c.c. 0·1 N HCl.	NH_3 in Urine c.c. 0·1 N.
1	0	410
2	0	538
3	0	485
4	0	550
5	0	422
6	770	770
7	770	787
8	0	710
9	0	667
10	0	704.

Assuming the weight of the subject to be 70 kg., the dose of acid per kg. is 11 c.c. 0.1 N HCl. Average ammonia excretion per day for the control period is 480 c.c. 0·1 N. If it is assumed that NH_3 would have been excreted at that rate for the rest of the period except for the acid given, increased ammonia excretion works out at 1233 c.c. 0·1 N; this is 80% of the acid given. But it is to be noted that even on the 10th day, three days after the acid administration was stopped, the ammonia excretion had not returned to normal. So the experiment is incomplete for our present purpose.

Increased NH_3 excretion on the 1st day is 290 c.c. 0.1 N, that is, 38% of the acid given.

NH_3 excretion rises to its highest on the second day of acid administration, and amounts to 317 c.c. 0·1 N in excess of the daily average for the control period. It is 39% of the acid given.

Dunlop (1896) studied ammonia mechanism in three human subjects - all three hospital patients. Only in one case did he make determinations both for ammonia and fixed base. This case of his is extracted in full.

Subject a chronic case of syphilitic paraplegia, weighed 62·6 kg. Throughout the experiment he was placed on a constant diet of:-

		Total Nitrogen in gms.
Oatmeal	100 gm.	2·33
Rice	100	1·24
Butter	20	·05
Cheese	40	1·78
Milk	100	2·35
Biscuits	225	2·99
Sugar	40	0·00
Vimbos	5	·89
Tea Tabloids	..	0·00
Mince Callops	100	6·12 / 17·75

After a preliminary period of three days he was given 648 c.c. of 0·1 N HCl a day for two days. The total dose for each day was given in three equal portions

before each meal.

Author's figures converted into terms of decinormal solutions are:-

	c.c. 0·1 N				Total N. gm.
	NH_3	K	Na	Sum	
Daily average for the fore-period of three days	542	417	1250	1667	12·23
1st day acid given 648 c.c. 0·1N HCl, i.e. 11 c.c. per kg. wt.	865	Daily Average. 300	1535	1835	12·95
2nd day. do.	935				
No after-period.					

Thus there was an excess of 323 c.c. of 0·1 N Ammonia on the first day and an excess of 393 c.c. of 0·1 N c.c. Ammonia on the second day; that is 50% and 60% respectively of the acid given, and 55% for the two days taken together. There is also an increased excretion of 336 c.c. 0·1 N fixed base for the two days, i.e. 26% of the acid given.

The second subject was a case of diarrhoea, and was placed on a similar mixed diet as reported above. After a fore-period of three days he received 11 c.c. of 0·1 N HCl per kg. weight for two days. Increased Ammonia excretion amounted to 20% of the acid given.

The third subject was a case of cardiac disease, placed on a constant mixed diet, received, after a fore-period of four days, 14 c.c. 0·1 N HCl per kg. body weight. The increased ammonia excretion amounted to 100 % of the acid given.

Limbeck (1898) in a general paper on acidosis gives the results of two experiments on human subjects of acid administration - in one case lactic acid was given and in the second case HCl. Particulars of the second experiment are as follows:-

Subject weighed 55 kg. and was kept on a constant diet of Milk 600 c.c., white bread 200-210 gm., Ham(lean) 150 gm., Roast Veal 100 gm., Beer 750 c.c., Rice 300 c.c., Soup 300 c.c. After a fore-period of three days, 1100 c.c.'s of 0·1 N HCl was given during the next three days divided into three equal portions. This was followed by an after-period of two days.

The author's figures for the experiment are:-

Days	Acid given 0·1N c.c.	Total N gm.	N (NH_3) ·1 N c.c.	Kcl + NaCl gm.	CaO gm.	Cl gm.	P_2O_5 gm.
1	0	11·94	500	25·43	·21	7·12	2·33
2	0	11·05	550	20·95	·19	4·13	6·67
3	0	13·86	605	15·75	·20	5·03	2·46
4	370c.c.	11·33	516	44·83	·146	4·45	2·06
5	370c.c.	13·84	700	44·28	·052	5.77	2·53
6	370c.c.	15·28	720	28·29	·031	6·89	2·87
7	0	15·78	663	20·71	·58	6·17	2·55
8	0	11·22	547	14·83	.49	2·79	2·12

It will be seen that for a dose of 7 c.c. ·1 N HCl per kg. weight, there is no increase in NH_3 excretion on the first acid day, while there is an increase of 54% of the acid given on the 3rd day and a total increase for the experiment of 38%. It is to be particularly noted that there is an increase in fixed base equivalent to about 400% of the dose of acid. No one before or since has reported anything even remotely resembling this.

The author comparing his results with results of experiments on dogs by Gaethgens came to the conclusion that in man ammonia production for neutralisation of acid did not play as important a rôle as in carnivora.

Camerer's (1902) subject was a youngman of 17, and to him he gave, after a preliminary period of one day, 300 c.c. of 0·1 N HCl a day for two days. Thereafter follows an

after-period of four days. The subject was on a mixed diet to which he added an extra amount of potatoes and green salad on the days on which he gave acid. His figures are:-

Days.	HCl 0·1 N.	T.N. gm.	NH_3 c.c. 0·1 N
1	0	14·43	590
2	300 c.c.	13·10	610
3	300 c.c.	13·58	710
4	0	11·88	545
5	0	11·80	460
6	0	12·55	500
7	0	11·61	440

The author comes to the conclusion that very little of the acid was neutralised by ammonia. This is the case if the increase is judged on the very inadequate fore-period of one day; thus there is an increase of 20 c.c. 0·1 N on the 1st day of acid period and an increase of 120 c.c. on the second day, i.e. ·66% and 40% respectively of the acid given. But if the increase be calculated on the basis of excretion of ammonia in the after-period, the extra ammonia comes to about 60% of the acid fed.

Camerer claimed his experiment to confirm the one previously reported by Limbeck. The two together evidently form the basis for the belief, long held and even now not

entirely discarded, that ammonia production is less important in man than in carnivora.

Eppinger (1906) experimented on dogs and rabbits to ascertain the effect of high and low protein diet on ammonia excretion after acid administration. He found that both dogs and rabbits succumbed to acid when on low protein diet. But it is to be noted that he employed very large quantities of acid, as much as 250 c.c. 0·1 N per kg weight. He further found that dogs became immune to acid when given high protein diet, while rabbits could stand much larger doses on food rich in protein. He concluded that there was very little, if any, difference between carnivora and herbivora as regards their reaction to acid administration, any apparent difference being due to the nature of the food.

Four of his experiments are extracted below:-

1. Dog, weight 4·89, on 350 gm. meat a day, given 1225 c.c. 0·1 N HCl ($\frac{3}{4}$ by mouth and $\frac{1}{4}$ subcutaneously) i.e. 250 c.c. 0·1 N HCl per kilogram, on the 4th day. The experiment was continued for a further period of four days. 244 c.c. 0·1 N NH_3 was excreted in excess of the average daily excretion for the period on the 1st day and 985 c.c. 0.1 N NH_3 in excess for the whole of the period; that is, 31% and 81% respectively of the acid given.

2. Dog weight 4·7 gms., fed on 90 gm. meat and 5 40 gm. lard daily. Fore-period three days. After-period four days. Excreted 250 c.c. 0·1N NH_3 on the first day, and 570 c.c. 0·1N NH_3 on the whole in excess of the average daily excretion for the fore-period, that is, 21% and 47% of the acid given.

3. After 5 days of starvation, the dog received 215 c.c. 0·1N HCl per kg. weight and this proved fatal. A similar dose given to rabbits under similar conditions also proved fatal.

4. Rabbit weighing 2·48 kg. on a diet of carrots and hay, when given 625 c.c. of 0·1N HCl (i.e. 250 c.c. per kg. weight) plus 15 gm. glycocol per day, could stand the intoxication quite well, and excreted 75 c.c. 0·1N NH_3 in excess on the first day, and 218 c.c. 0·1N in excess on the whole; that is, 12% and 34·8% of the acid given.

Pohl and Münzer (1906) repeated Eppinger's experiments on rabbits using his mode of administration and used glycocol or urea with the acid but in every case their rabbits died. They mention that one rabbit which had received only half of the dose usually used by Eppinger and had received 15 gm. glycocol died, while a control which received the acid but no glycocol survived. They denied that urea or glycocol gave any protection against acid. But it is to be noted that they gave urea by the mouth, while Eppinger had given it

it in every case subcutaneously. In their experiments with glycocol, though they did give glycocol subcutaneously, they gave as much as 330 c.c. ·1 N HCl per kg. weight, while Eppinger never gave more than 250 c.c. 0·1N HCl per kg weight.

Eppinger and Tedesko (1909) returned to the subject again because Pohl had failed to confirm Eppinger's results. Pohl had also pointed out that death of dogs in Eppinger's previous work was due to starvation. In the present work, he repeated his previous experiments by keeping his animals on a carbohydrate diet, and thus eliminating the effect of mere hunger.

1. Dog, weight 9·8 kg., on carbohydrate diet, after a preliminary period of four days in which NH_3 excretion fell from 560 c.c. 0·1N NH_3 to 160 c.c. 0·1N c.c. NH_3, and total nitrogen from 3·37 gm. to 1·97 gm., received 1500 c.c. 0·1N HCl, i.e. 150 c.c. per kg weight. The animal died, but in the hour before it died it produced 79 c.c. 0·1 N NH_3 in excess, i.e. 2% of the acid given.

2. Dog on meat diet, received 250 c.c. 0·1N HCl per kg. weight, produced on the first day NH_3, in excess to neutralise 51% of the acid given. Excess of NH_3 for the whole experiment amounted to 63% of the acid given. Increased fixed base excretion amounted to 51% of the acid given.

3. A sheep weighing 16 kg. received for the first four days of the experiment carrots and bread, and its total N excretion and NH_3 excretion for the fourth day was 4·7 gm. and 200 c.c. 0·1N respectively. On the fifth day it received 60 gm. plasmon in addition to its usual ration and for the next four days 120 gm. plasmon were given with the usual rations. Excretion for the ninth day was T.N 12.23 and NH_3 765 c.c. 0·1N. The average NH_3 excretion amounted to 440 c.c. 0·1N. per day.

On the tenth day the animal received 4000 c.c. 0·1N HCl (e.i. 250 c.c. 0·1N HCl per kg). Increased ammonia excretion amounted to 2000 c.c. 0·1N in excess of the average fore-period excretion on the first day and 3720 c.c. 0·1N for the whole period (i.e. including two days after-period). Thus the increased NH_3 excretion amounted to 50% on the first day and 93% on the whole.

4. A sheep on purely carbohydrate rations for a period of six days, when it received 107 c.c. 0·1N HCl per kg. of its weight, ~~it~~ died in seven hours. During these seven hours it produced an excess of NH_3 amounting to 14% of the acid given.

The authors maintained that even under conditions in which hunger was excluded, but food lacking protein was used, acid considerably less than 250 c.c. 0·1N per kg. (150 c.c. in one case and 107 c.c. in another) proved fatal both to herbivora and carnivora. But the addition

several weeks of starch feeding, and studied the effects of acid and alkaline salt mixtures, and free mineral acids on endogenous nitrogen metabolism.

In a pig weighing 18 kg. while fed on starch and acid salts, NH_3 excretion amounted to 320 c.c. 0·1N and T.N. to 1·55 gm. a day. The substitution of alkaline salts 12 gm. a day dropped T.N. to 1·09 gm. and NH_3 excretion to 52 c.c. 0·1N a day. Urea N and creatinine N remained constant.

They concluded that during the first period additional amount of protein had been catabolised in response to the acid character of the food. They made the deduction that the organism was not able to utilise the nitrogen of urea fraction to form ammonia to neutralise the acidity and thus prevent an increased nitrogen elimination. To demonstrate this point more fully they exhibited HCl. To a young pig weighing 46·3 kg. they gave 2750 c.c. 0·1N HCl (i.e. 59·5cc. per kg. weight) a day for five days. T.N. excretion increased from an average of 2·86 gm. to 4·03 gm., while no change was observed in urea N and creatinine N. Protocol of this experiment is given below:-

Pig - wt. 46·3 kg.

Daily Ration	Period	Day	TN. gm.	Urea N. gm.	NH_3 c.c.0·1N
Starch + Benzoic Ac.16 gm.	IV	Daily average	2·86	0·55	274
Starch + 2750 c.c. 0·1N HCl + Benzoic Ac.16 gm.	V	28th	3·96	0·57	815
		29th	3·96	0·47	885
		30th	3·88	0·62	910
		31st	4·28	0·43	1275
		32nd	4·09	0·53	1275
		Daily average	4·03	0·54	1170

Day	Acid given c.c. 0·1N	Increase in NH_3 c.c. 0·1N	Increased NH_3 as Percentage of acid given
28	2750 @ 59·5c.c.perkg.	541	19·7%
29	2750	611	22·2%
30	2750	636	23·0%
31	2750	1001	36·4%
32	2750	1001	36·4%
Total	13750	3790	27.5%

It is to be noted that they used a growing pig and have failed to consider the question of growth when drawing conclusions from their observed facts.

Secchi (1914) gives figures both for fixed base and ammonia in urine of dog and man after administration of acid:-

I. Dog, 14 kg. weight. Daily food: 400gm. horse flesh, 150 gm. white bread, 2 gm. bone powder, 600 c.c. water - diet rich in calcium.

	NH_3 c.c. 0·1N		K+Na gm.	CaO gm.	MgO gm.
Fore-period 7 days - daily average	410		2·45	0·085	0·092
Acid period: 1st day 630 c.c.0·1N HCl, i.e. 45 c.c. per kg.	570	) av.	3·65	0·13	0·12
2nd day 630 c.c. do.	645	)			
After-period: 1st day	660	) av.	2·45	0·10	0·10
2nd day	720	)			

Increase in NH_3 on 1st day 160 c.c. 0·1N, that is 25·4% of the acid given.

Increase in NH_3 for the whole experiment 955 c.c., that is 76% of the acid given.

Increase of 2·5 gm. K+Na, CaO and MgO, roughly about 18% of the acid given on the first day.

II. Dog, 24 kg. wt. Daily food: 800 gm. horse flesh, and water - no bone powder, Ca low:-

	NH_3 c.c. 0·1N	K + Na gm	CaO gm	MgO gm.
Fore-period: 3 days daily average	945	3·35	0·064	0·125
Acid period:				
1. 512 c.c. 0·1N HCl	1023	3·95)		
2.1024 do.	2047	5·41)	av.0·073	0·20
3.1536 do.	2700	4·96)		
After period:				
1	1890			
2	1270			
3	1260			

Increased NH_3 excretion on the 1st acid day: 78 c.c. 0·1N, that is, 15% of the acid given.

Increased NH_3 excretion for the whole experiment 2630 c.c. (or more), that is 85.5% of the acid given.

III. Man, a case of gastric cancer, weight 65 kg. Daily diet: 200 gm. milk, 2 eggs, 600 c.c. water.

	NH_3 c.c.0·1n	K+Na gm.	CaO gm.	MgO gm.
Fore-period, 3 days daily average	350 c.c.	4·66	0·35	0·11
Acid period:				
1st day 480 c.c.0·1N HCl	560	5·25	0·47	0·12
2nd day 635 do.	700	6·19	0·37	0·13
3rd day 1000 do.	740	5·60	0·42	0·13
After period: 3 days daily average	540	4·47	0·40	0.09

Increased NH_3 excretion 1st acid day: 115 c.c. 0·1N, that is 24%.

Increased NH_3 excretion for the whole experiment 2115 c.c. 0.1N, that is 72% of the acid given.

Base shows an increase of about 18% of the acid given.

Secchi's experiments illustrate well the progressive rise in NH3 excretion. It is also to be noted that the excretion of fixed base rises quickly, but subsides long before the rise in NH_3 excretion. Owing to the fact that all experiments were stopped before NH_3 returned to the level of the fore-period, the experiments are not very valuable.

Steenbock, Nelson and Hart (1914) studied the question of acidosis in relation to protein storage in pigs and calves.

Animal No.4 - Calf: wt. 56·7 kg.

Ration - 12 lbs. whole milk daily.

	T.N. gm.	NH_3 c.c. 0·1N
Fore-period: 4 days, daily average	9·10	293
1st day, Acid 1920 c.c. 0·1N HCl @ 33·8 c.c. per kg.	11·38	775
2nd day do.	10·25	1020
3rd day do.	8·85	1100
4th day do.	10·93	1260
5th day do.	10·99	1220
After-period:		
1st day	8·43	1200
2nd day	9·34	1002
3rd day	10·26	750
4th day	8·61	450
5th day	10·68	470
6th day	9.28	451

1st day increase in NH_3 excretion 482 c.c., that is 25% of the acid given.
Total increase in NH_3 excretion 9600 c.c., that is 60% of the acid given.

Calf, wt. 56·7 kg. Low Nitrogen intake.

Daily Averages.

Periods	HCl C.C.O.IN	Rations	Urinary N	Urinary NH_3	P fed	P faeces	P Urine	P Balance
			gm	C.C.O.1N	C.C.O.IM	C.C.O.IM	C.C.O.IM	C.C.O.IM
Fore period of 5 days	0	3 lbs Straw 2 lbs Starch and Salt	6.71	620				
Acid period 6 days	92	2 lbs Straw 2 lbs Starch and Salt	7.06	1100				
Acid period 7 days	2000	1 lb Straw 1 lb Starch	6.48	- 850	670	49	253	+ 368
Acid period 3 days	3000	1 lb Straw 1 lb Starch	6.54	1070	670	70	332	+ 168
21st day	4000	do.	6.94	1700	670	54	280	+ 336
22nd day	4000	do.	7.43	1800	670	48	255	+ 367
23rd day	4000	do.	6.90	1800	670	45	163	+ 462

It will be observed that at this high level of protein intake, the administration of acid causes a great increase in NH_3 excretion, but accompanying this there is a fall in the output of urea, and except in one case of Steenbock's experiments, there is no increase in the total N excretion. These observations are contrary to the findings of McCollum and Hoagland. But of course their animals were on starch diet for long periods before acid was given and nitrogen excretion had fallen to the minimum, and again the animals received large doses of acid for long periods. Steenbock and others found practically the same results with herbivora as with omnivora. This is in keeping with the work quoted above, that herbivora on high protein intake can control acidosis by NH_3 production like carnivora.

They also report experiments with calves on low protein intake. Here, too, acid administration leads to increased NH_3 excretion, though not to the same extent as in the experiments cited above. No increase in T.N. was observed, and the increased NH_3 excretion was compensated by decreased Urea excretion. They also determined ammonia content of the fæces before and after acid administration and found it to be constant and came to the conclusion that ammonia on low protein intake was formed in the body tissues, though some must be formed by putrefaction processes in the bowels when the animals are on high protein intake.

Protocol is given below:-

Animal No.3. Calf - 50 kg. wt.

Date	Daily Averages											
	Vol. Urine	Intake N	Faecal N	Urinary N	Urinary NH3 N	Urea N	Percent NH3 N	Percent Urea N	Sum per cent	N Excreted	N Retained	Percent N Retained
	c.c.	gm	gm	gm	gm	gm				gm	gm	
	14 lbs whole milk.											
Nov. 17-22	4197	35.11	2·35	11.18	0·50	8·50	4·5	76·1	80·6	13·53	21·54	61·3
	14 lbs whole milk + 16 c.c. NHCl per lb milk.											
23-28	4454	35.11	1.97	14.53	2.46	9.56	17.1	66.1	83.2	16.50	18.61	53.0
	14 lbs whole milk + 16 c.c. NHCl per lb milk.											
Nov-Dec. 29-4	4581	35.11	1.46	12.60	3.34	7.21	26.6	57.1	83.7	13.65	21.04	59.9
	16 lbs whole milk + 16 c.c. NHCl per lb milk.											
Dec. 5-12	5397	40.13	1.59	14.20	3.50	8.31	24.7	58.6	83.3	15.79	24.32	60.5

Begun, Hermann and Münzer (1915) give figures for NH_3 and P. excretion in urine after acid administration for two men. In the first case a constant diet of bread 21dkg., butter 8dkg., 7 eggs, meat 16dkg., rice, milk 250c.c., 12 pieces sugar, 3 cups of tea and 2 oranges were given and after a fore-period of nine days dilute HCl was given for a period of ten days. Results are given below:-

	T.N. gm.	NH_3 c.c. 0·1N	N (NH_3) in % of N.	P 0·1M c.c.
Fore-period of 9 days Daily average	13·78	447	4·50	312
Acid period: 10 days				
Daily dose of acid 576 c.c. 0·1N HCl, assuming wt. of subject to be 70 kg., about 8 c.c. per kg.wt.				
Daily average	15·10	855	7·89	356
After-period:				
Daily average	15·04	676	6·17	320

Increase of NH_3 excretion on the first day 98 c.c. 0·1 N, that is 17% of the acid given.

Total increase in NH_3 excretion 4430 c.c. 0·1N, that is 61%.

Second subject - practically the same diet as given to the first subject:-

	T.N. gm.	NH_3 c.c. 0·1N	N (NH_3) % of N.	P.
Fore-period of 3 days Daily average	13·50	541	5.65	270
Acid period, 3 days 960 c.c. 0·1N HCl daily, assuming wt. to be 70 kg., dose per kg.weight about 14 c.c. 0·1N HCl.				
Daily average	13·65	935	9·58	303
After-period, 3 days				
Daily average	13·39	800	8·76	271

Increase in NH_3 excretion on 1st day, 189 c.c. 0·1N, that is 20% of the acid given.

Increase in NH_3 excretion for the whole experiment 1960 c.c. 0.1N, that is 68% of the acid given.

Stehle (1917) used dog for his experiment to study the effect of acid administration on base excretion.

Dog: weight 9 kg. A constant diet of 50 gm. soda crackers, 150 gm. beef heart, 10 gm. agar-agar. After a fore-period of three days on this diet, a period of another three days followed during which glycocol HCl 9 gm. a day was given. After this 4925 c.c. of 0·1N HCl were given during the next twelve days - 1 gm. HCl doses for the first four days, and 2 gm. HCl doses during the rest of the period with no acid on one day. During the period of mineral acid administration 2910 c.c. 0·1N NH_3 and 853 c.c. 0·1N fixed base were excreted in excess. In other words, NH_3 neutralised 57% of the acid given and fixed base 17%. For lack of after-period and the varying dosage of acid administration it is not possible to arrive at any really accurate estimate of the base excretion and above figures are given for what they are worth.

Marriott and Howland (1918) used five hospital patients. They were all on normal hospital diet, but the composition and amounts of the diet are not given.

1st Experiment:

	T.N. gm.	NH_3 c.c. 0·1N	P. c.c. 0·1M.
Daily average for a fore-period of 3 days	12·3	345	274
4th day 500c.c. 0·1N HCl	12·6	448	357
5th day, after-period	13·5	350	264

Increased NH_3 excretion = 103 c.c. = 20% acid.

2nd Experiment:	T.N. gm.	NH_3 c.c.0·1N	P. c.c. 0·1M
Daily average for a fore-period of 3 days	13·10	382	297
4th day 500 c.c.0·1N HCl	12·00	504	86276
5th day, after-period	12·30	376	90290

Increased NH_3 excretion = 122 c.c. = 24% of acid.

3rd Experiment:	T.N. gm.	NH_3 c.c.0·1N	P. c.c. 0·1M
Daily average for a fore-period of 2 days	16·3	454	433
4th day 500 c.c. 0·1N HCl	16·9	566	420
5th day, after-period	16·6	560	400

Increased NH_3 excretion = 228 c.c. = 44% of acid.

4th Experiment:	T.N. gm.	NH_3 c.c. 0·1N	P. c.c. 0·1M
Daily average for a fore-period of 3 days	12·2	431	330
4th day 500 c.c. 0·1N HCl	12·4	596	300
5th day, after-period	9·5	460	329

Increased NH_3 excretion = 194 c.c. = 39% of acid.

5th Experiment:	T.N. gm.	NH_3 c.c. 0·1N	P. c.c. 0·1M.
Daily average forafore-period of 3 days	-	382	-
4th day 500 c.c.0·1N HCl	-	529	-
5th day, after-period	-	439	-

Increased NH_3 excretion = 204 c.c. = 41% of acid.

It is to be noted that after-period of one day is too short, as in three experiments out of the five quoted NH_3 excretion had not returned to normal when the experiments were stopped.

Lamb and Evvard (1919) give the results of experiments on pigs; these are interesting for the striking progressive increase in NH_3 excretion which they show.

1st Experiment: Pig, weight 27·4 kg. on a feed rich in calcium content - 1·12% Ca.

	T.N. gm.	NH_3 c.c. 0·1N
Fore-period of 10 days Daily average	10·37	1001
Acid period, 10 days - 3000 c.c. 0·1N H_2SO_4 daily @ 110 c.c. perkg.		
1.	9·603	1530
2.	9·541	1737
3.	10·534	2240
4.	11·259	2320
5.	11·647	2480
6.	11·293	2540
7.	12·912	3000
8.	12·139	3070
9.	9·235	2500
10.	15·812	4650

	T.N. gm.	NH_3 c.c. 0·1N
After period - 8 days		
1	Daily average for N	3340
2	for after-period	2730
3	11·61	1570
4		1520
5		1200
6		1112
7		1110
8		1140

Total increased NH_3 excretion = 21789 c.c. 0·1N = 73% of acid given.

1st day - increased NH_3 excretion = 529 c.c. 0·1N = 17% of acid given.

10th day - increased NH_3 excretion = 3649 c.c. = 121% of acid given.

2nd Experiment: Pig wt. 65 kg., on a ration of low Ca content - 0·022 %.

	T.N. gm.	NH_3 c.c. 0·1N
Fore-period- 5 days		
Daily average	10·840	1140
Acid period - 3000 c.c. 0·1N H_2SO_4 daily @ 625 c.c. per kg.		
1	12·197	1670
2	10·533	2200
3	12·567	3300
4	11·883	3290
5	12·132	3240
6	10·607	2910
7	12·678	3340
8	13·405	3510
9	10·997	3260
10	13·368	3730

	T.N. gm.	NH_3 c.c. 0·1N
After-period - 5 days		
1	14·218	2270
2	11·970	1680
3	10·269	1300
4	11·029	1290
5	8·399	870

Increased NH_3 excretion for the whole experiment = 21760 c.c. 0·1N = 73% of acid given.

Increased NH_3 excretion 1st day of experiment = 530 c.c. 0·1N = 18%

Maximum increase in NH_3 excretion - 10th day of experiment - 2590 c.c. 0·1N = 86%.

Stehle and McCarty's (1921) experiments designed to elucidate some facts of Ca metabolism are quoted below as they give figures for NH_3 and fixed base excretion after acid administration. They used themselves as the experimental subjects and kept themselves on a constant diet of rice, French fried potatoes, bananas, wheat bread, jam, butter, apple-pie and figs.

1st Experiment:

After a fore-period of four days - 1000 c.c. 0·1N HCl was taken daily for three days - no after period. Although the fore-period lasted four days, determinations were carried out for the last two days only.

	Day of Expt.	T.N. gm.	NH_3 c.c.0.1N	Na c.c.0.1N	K c.c.0.1N	Ca c.c.0.1N	Mg c.c.0.1N	P c.c.0.1M	pH
Fore-period	3	6·85	173	610	620	29	87	113	6·70
	4	6·59	150	430	510	37	96	120	6·75
Acid period	5	7·95	328	890	1170	45	150	168	6·28
1000 c.c. 0·1N HCl daily	6	7·34	390	700	840	45	132	174	5·27
	7	6·97	430	700	960	56	134	173	5·05

(Original figures converted into terms of decinormal solutions).

Total acid given = 3000 c.c. 0·1N HCl

Total excess of NH_3 excreted = 662 c.c. 0·1N = 22%

Total excess of fixed base excreted = 2205 c.c.0·1N,i.e. 73% of acid given.

1st day:

Acid given = 1000 c.c. 0·1N HCl.

Excess NH_3 = 166 c.c. 0·1N, i.e. 17%.

3rd day:

Acid given = 1000 c.c. 0·1N HCl

Excess NH_3 = 268 c.c. 0·1N, i.e. 27%

2nd Experiment:

The same diet, and the same quantities of acid as in the 1st Experiment. Fore-period of acid days also the same.

	Day of Expt.	T.N. gm.	NH_3 c.c.0·1N.	Na c.c. 0·1N	K cc.0·1N	Ca c.c.0·1N	Mg. c.c.0·1N	P. c.c.0·1M	pH
Fore-period	3	6·27	233	610	360	36	87	86	5·86
	4	6·16	177	630	550	50	79	99	6·44
	5	6·42	300	775	710	70	120	129	6·89
Acid period 1,000 c.c. 0·1N HCl daily	6	6·29	430	730	800	61	110	156	5·05
	7	6·29	446	560	800	85	100	167	5·00

Total acid given =3000 c.c. 0·1N HCl.

Total excess NH_3 excreted - 561 c.c. 0·1N = 17%

Total excess fixed base excreted = 1328 c.c.0·1N, that is 44% of the acid given.

1st day:

Acid given 1000 c.c. 0·1N HCl.

Excess NH_3 excreted = 95% = 9·5%

2nd day:

Acid given 1000 c.c. 0·1N HCl

Excess NH_3 excreted = 241 c.c. 0·1N, i.e. 24%

Since the amounts of the various articles composing the diets used are not given, it is not possible to say definitely, but from low NH_3 excretion in the fore-period, as compared with several human experiments quoted above, and the high pH values for urine, suggest that the diets had a high alkali content. This would explain the low NH_3 excretion after acid administration.

Zucker (1921) gives the following figures for NH_3 excretion after acid administration to a human subject:-

Days. Fore-period.	NH_3 c.c. 0·1N.
1	1014
2	650
3	860
4	860

Then follows a period of three days during which 15 gm.. of Na H Co_3 were given per day.

Days. Acid period:		NH_3 c.c. 0·1N
7	300 c.c. 0·1N HCl	435
8	300 c.c. 0·1N HCl	780
9	300 c.c. 0·1N HCl	1030
10	300 c.c. 0·1N HCl	1180

If the acid day immediately following the NaH Co_3 be ignored the increased NH_3 excretion amounts to 520 c.c. 0·1N for 900 c.c. of 0·1N HCl given for that period, that is 57%.

There is an increase in NH_3 of 100% of the acid given on the 4th day.

There is no after-period, so the figure for the total excretion is only approximate.

Keeton (1921) quotes from literature to show that ammonia excretion is not a mechanism which is called into play consistently by the human organism under all conditions in which it might be supposed in reason that every available means of protection should be employed. He contrasts the high ammonia excretion in diabetes, cholera and certain diarrhoeal diseases of children with the low ammonia excretion in uraemia acidosis. He observes that it is the production of acetone bodies in disease which call forth increased ammonia excretion and these are believed to be formed largely in the

liver. "If it be granted that the phenomenon of neutralisation of acids by ammonia has been observed with the greatest consistency and in the highest degree in two conditions: (a) a type of acidosis in which the acids are largely hepatogenous in origin, and (b) a type following the administration of acids, but that the phenomenon is found to be less marked or entirely absent in other types of acidosis, the question would arise as to why the ingestion of acid should produce an effect like that of one type of acidosis and unlike that of other types. Could this mean that in order to be neutralised to the highest degree by ammonia the free acids involved must be formed within or introduced into the liver?"

Keeton notes that in literature in all cases in which increased excretion of ammonia was observed, the acid had been given by the alimentary route. Keeton very rightly rejects Winterberg's (1898) experiments as of little value. He administered acid subcutaneously and noted increased ammonia excretion. NH_3 increase noted is comparatively insignificant, and even as such NH_3 figures given are much too high. On a carrot diet the rabbits do not show ratio of $\frac{NH_3\ N}{T.N.}$ higher than about 0.1%, while Winterberg's results are six times that figure. Winterberg's protocols are not reproduced because they have no value as at the time no accurate method of ammonia determination when present in small quantities was known.

Keeton, therefore, undertook experiments for the purpose of comparing the relative effect on the ammonia excretion in urine of acid administration by the alimentary route and by the peripheral vein. Alimentary administration should subject the liver to the effects of a relatively large quantity of free acid, while the latter would afford opportunity for the neutralisation of more acid in the blood or tissues outside the liver.

After acid by the mouth he finds an absolute increase in ammonia nitrogen, without change in the total nitrogen excretion. After acid by the vein the total N excretion rises considerably, and the ammonia nitrogen though it follows the rise of T.N. closely does not show an absolute increase. The rise in total N. after intravenous administration he interprets to mean that there is a definite toxic destruction of protein induced by acid injection, and increased ammonia figure under these conditions is, he holds, merely an acceleration of normal proteolysis.

Results.

I. By the alimentary route.

Dog 1, wt. 16·17 kg. Diet: Milk 100-200 c.c., bread 50-100 gm sodium acetate, 1 gm. daily.

1.	T.N.	NH_3 c.c.0·1N
Fore-period 3 days Daily average	5·43	228
4th day, acid given 125 c.c.0·1N HCl	5·57	358

Increased NH_3 secretion = 130 c.c. = 100+%

	T.N. gm.	NH_3 c.c. 0·1N
2. Fore-period 3 days Daily average	4.66	196
4th day 200 c.c.0·1N HCl	4.40	257

Increased NH3 excretion = 61 c.c. = 30·5%

Dog 5. wt. 17·8 kg. Same diet as above.

1. Fore-period 3 days Daily average	4·41	179
4th day 180 c.c. 0·1N HCl	4·72	244

Increased NH_3 excretion = 50 c.c. = 27% (authors)

2. Fore-period 3 days Daily average	4·35	179
4th day acid 250 c.c. 0·1N HCl	4·53	283

Increased NH_3 excretion = 104 c.c. = 42%

II. Acid administration by vein.

Dog 1, wt. 16·17. Same diet as before.

	T.N. gm.	NH_3 c.c.0·1N	(NH_3) N. as % of T.N.
1. Fore-period 3 days Daily average	5·63	212	·58
4th day acid 125 c.c.0·1N HCl	6·93	300	·62

Increased NH_3 = 88 c.c. = 70%

2. Fore-period 3 days Daily average	3·95	165	·59
4th day 200 c.c. 0·1N HCl	5·57	164	·39

No increase in NH_3 excretion.

3. Fore-period 3 days. Daily average	5·48	212	·62
4th day 200 c.c.0·1N HCl	6·94	210	·46

No increase in NH_3 excretion.

Dog. 5. wt. 17·8 kg.	T.N. gm.	NH_3 c.c.0·1N	$(NH_3)N$. as % of T.N.
1. Fore-period 3 days Daily average	4.10	150	·53
4th day, 180 c.c. 0·1N HCl	5·70	212	·52
Increased NH_3 excretion = 62 c.c. = 34%			
2. Fore-period 3 days Daily average	4·73	229	·67
4th day, 250 c.c.0·1N HCl	6·24	271	·60
Increased NH_3 excretion = 42 c.c. = 17%			

After alimentary administration, 100+ %, 31% 27% and 42% respectively of the acid given is neutralised by NH_3.

After intravenous administration, 70%, 0%, 0%, 34% and 42% respectively of the acid given is neutralised by NH_3.

The author argues that after intravenous administration there is a rise in T.N. excretion and if allowance is made for the increased NH_3 production which would result from normal proteolysis represented by the increased T.N. excretion, there is no increased NH_3 excretion for neutralisation. There is no doubt that there is a definite toxic destruction of protein induced by acid injection. The secondary changes thus induced, so upset the organism as a whole, that the intravenous injection experiments of Keeton do not afford any reliable information on the subject of NH_3 production and the conclusions drawn by him are not valid.

Bayliss (1919),before Keeton,had noted that however slowly the acid was run into the vein (of a cat) some degree of haemolysis resulted. Sizili (1906) noticed haemolysis in sheep after acid administration. There is no doubt that free Hb injures the kidney; it does not matter how temporary the injury it can not leave the function of the kidney normal. Even if haemolysis is not noticed, as perhaps Keeton did not in the case of dogs, the increased T.N. excretion is evidence of profound secondary changes induced by the acid.And if the view of Nash and Benedict (1921) be taken as accurate, that ammonia production takes place largely in the kidney, it would be easy to see that injury to the kidney must interfere with NH_3 production to a great extent.

(2) Phosphate Excretion.

Gerhardt and Schlèssinger (1899), as evidence that there is an excretion of P^{1} in excess of that accountable by protein catabolism, report a $\frac{P}{N}$ ratio of $\frac{3{\cdot}9 - 4{\cdot}4}{100}$ in the urine of two diabetics with severe acidosis. The normal ratio under similar conditions was estimated at $\frac{2{\cdot}7}{100}$. Feeding of sodium bicarbonate to the diabetics reduced the ratio to $\frac{3}{100}$. Rumpf (1898) and Mendel and Lusk (1904) report in a fasting patient with severe diabetes $\frac{P}{N}$ ratios similar to that of Gerhardt and Schlessinger. Folin and Shaffer (1902), however, found $\frac{3{\cdot}9 - 4{\cdot}4}{100}$ ratio common in normal adults.

The experiments of Fitz, Alsburg and Henderson (1907) on rabbits confirmed the belief, which is still widely held, that increased phosphate excretion plays an important rôle in the elimination of acids from the body. They observed a marked increase in the phosphate excretion by kidney after acid administration. The average for four

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1. The figures for phosphate expressed in different ways in the various works quoted have been converted and expressed in terms of P, in this paper.

rabbits for the first fortnight of the experiments was an increase of 55% on the fore-period excretion. This increase was eventually followed by a period of diminished excretion, excretion falling below that in the fore-acid period. This decrease they interpreted as signifying exhaustion of the readily available phosphates in the body. They put forward the conclusion that the phosphates are intimately concerned not only with the neutralisation of acid within the body in experimental acidosis, but also in its removal from the body. The results of one of their experiments are reproduced for further analysis.

Rabbit; weight 1585gm.fed on a more or less constant feed of 300 gm. cabbage per day:-

Day of Experiment.	Weight.	Acid injected. c.c. 0·1N HCl.	P. 0·1M c.c.
1	1585	0	10·9
2		0	9·0
3		58	9·0
4	1550	do.	12·0
5		do.	9·6
6		do.	11·0
7		76	17·5
8		do.	20·0
9		do.	22·6
10		do.	10·8
11	1530	do.	23·0
12		do.	10·0
13		123	20·0
14		do.	10·0
15		do.	16·0
16		do.	9·0
17		161·5	lost
18	1440	do.	40·0
19		do.	9·0
20		do.	19·0
21	1335	do.	32·0
22	Died	do.	

This represents an increase of 140 c.c. 0·1M of P, that is 238 c.c. of the total acid given (1987 c.c. 0·1N) were eliminated by the kidney through increased phosphate excretion. This amounts to about 12% of the acid given.

It is unfortunate that the authors do not give figures for T.N. excretion, but it is to be noticed that the weight of this rabbit fell from 1585 gm. to 1335 gm. and it died on the 22nd day. The weights of the other three rabbits fell similarly and they all died. There can be little doubt that increased phosphate excretion is due to increased breaking down of tissues of the body. The repeated doses of 58 c.c. to 161 c.c. of 0·1N HCl given to rabbits of 1·5 kg. weight are too big, and the secondary results of acid administration leading to death must complicate results beyond hope of interpretation.

Steenbock, Nelson and Hart (1914) found that acid feeding to pigs and calves increased phosphate excretion in the urine. They made determination of phosphate in food and in faeces, and in no case did they find a negative balance, except when massive doses producing secondary results were given.

Calf wt. 56·7.For ration see page .

	HCl c.c. 0·1N	P.in food c.c.0·1M	P.in faeces c.c. 0·1M	P.in Urine c.c.0·1M	P.Balance c.c.0·1M.
Daily Av. 7 days	2000	670	49	253	+ 368
Daily Av. 3 days	3000	670	70	332	+ 168
21st day	4000	670	54	280	+ 336
22nd day	4000	670	48	255	+ 255
23rd day	4000	670	45	163	+ 163

Begun, Hermann and Münzer (1915) obtained the following results on two human subjects on a constant diet of bread 21 dkg., butter 8 dkg., eggs 7,meat 16 dkg., Milk 250 c.c., oranges 2, tea 3 cups, sugar 12 pieces,and rice.

	T.N.	P. 0·1M c.c.
1. Daily average for a fore-period of 9 days	13·78	312
Acid period 10 days 576 c.c. 0·1N HCl daily, daily av.	15·10	356
Daily average - after-period 3 days	15·04	320

For 5760 c.c. of 0·1N HCl, there is an increased excretion of 464 c.c. 0·1M c.c. P. In other words, 13% of the acid given was neutralised by phosphate.

	T.N.	P. c.c. 0·1M
2. Daily average for a fore-period of 3 days	13·50	270
Daily average - acid period 3 days 960 c.c. 0·1N HCl daily	13·65	303
Daily average - after-period 2 days.	13·39	271

For 1920 c.c. 0·1N HCl, there is an increased excretion of 66 c.c. of 0·1M P. 5·7% of the acid neutralised by phosphates.

Givens and Mendal (1917) give results of experiments on a dog.

Dog weighing 13·2 kg., constant diet of meat, dried bread, skimmed milk and agar agar. The composition of the diet was N 10 gm., Fat 51 gm., carbohydrates 51 gm., CaO 223 mg., MgO 170 mg., P 194 c.c. 0·1M.

410 c.c. of 0·1N HCl were given daily for a period of 3 days.

	P. in Urine c.c. 0·1M	P. in fæces c.c. 0·1M	P. Balance	N. in Urine gm.	N. in fæces in gm.	N. Balance gm.
1	108	15	+ 71	8·99	542	+ 0·56
2	148	16	+ 30	8·55	432	+ 1·10
3	72	26	+ 96	8·39	592	+ 1·12

Both P. balance and N. balance are positive right through the experiment. When determinations for P. content are done for both fæces and urine, it appears the increased

excretion of P. in urine is due to increased absorption of P. from the intestines, because acid renders the phosphates in the bowels more soluble.

Gotto (1918) in an experiment on rabbits confirmed the increased excretion of phosphates after acid administration as reported by Fitz, Alsburg and Henderson. But they suggested that such increase has two possible explanations; it may be derived from the tissues of the body to neutralise the acid, or it may be due merely to increased absorption of Ca and Mg phosphates of the food rendered more soluble by the ingested acid in the alimentary tract. Gotto, therefore, undertook the determination of phosphates both in urine and fæces.

Rabbits used weighed from 1400 gm. to 1530 gm.

P. in urine of normal rabbits, daily average, from 10·9 c.c. 0·1M to 15·3 c.c. 0·1M.

P. in fæces of normal rabbits, daily average, from 4 c.c. 0·1M to 28 c.c. 0·1M.

Acid given: 125 c.c. 0·1N HCl per day.

P. in urine for acid period, daily average, from 25 c.c. 0·1M to 44 c.c. 0·1M.

P. in fæces for acid period, daily average, from 8·3 c.c. 0·1M to 50 c.c. 0·1M.

The increased excretion of P. in urine, is not accompanied by a decrease in the P.content of the fæces . They came to the conclusion that the urinary increase indicated a genuine negative balance. This result is at variance with Given and Mendal's work. But it is to be noted that different sets of animals were employed for determining normal values and acid period values, as the author desired to carry out analysis for tissue P. contents. Also this author used very massive doses: 25 to 75 c.c. of 0· 25 N HCl a day for 13 to 38 days to rabbits weighing about 1500 gms. Unfortunately, the author does not give figures for T.N. excretion, but from the fact that every one of his experimental animals died from 7th to 38th day, it is to be surmised that secondary effects of his acid doses were profound.

From the work of Marritt and Howland (1918) quoted on page , it will be seen that in experiments 2, 3, and 4, there is no increase of P. excretion in urine after acid administration to human subjects, While in experiment 1, there is an increase of 83 c.c. 0·1M P. for 500 c.c. of 0·1N HCl given. All the subjects were on diet, and no figures are given for P. content of fæces .

Zucker (1921) gives figures for phosphate excretion in urine and fæces for a human subject. The subject was kept on a constant mixed diet right through the experiment.

	P. in urine c.c.0·1M	P.in fæces c.c.0·1M	Total P. c.c.0·1M	P percentage in Urine
Total for the last 3 days of a fore-period of 4 days	1730	1283	3013	57
Total for the 3 days on which 15 gm. $NaHcO_3$ a day was given	1450	1520	2970	44
Total for the last 3 days of a period of 4 days on which 300 c.c. of 0.1N HC. was given per day	1800	1150	2950	61

The P. is increased in urine at the expense of the fæcal P. and without loss to the body. This confirms Given and Mendel's work.

Haldane (1921) reported that in experimental acidosis there is increase in urinary phosphates. The subject was kept on a constant diet right through the experiment.

Day of Experiment	Acid given c.c. 0·1N HCl	P c.c. 0·1M
1	0	409
2	3740	4545
3	3740	662
4	2810	660
5	0	389
6	0	349
7	0	313

There is a slight increase in P. excretion, but as the subject was on diet, and no figures for P. content of fæces are given, the experiment quoted does not help the solution of the problem.

Haldane, Hill and Lusk (1923) give the following figures for NH_3 and P excretion in Calcium chloride acidosis:

Subject J.B. S.H. wt. 100 kilos.

	NH_3 c.c. 0·1N	P. c.c. 0·1M
Fore-period	46·4	39·8
Acid period:		
225 c.c. 0·1M $CaCl_2$	135·3	50·5
275 c.c. 0·1M $CaCl_2$	164·1	66·5
275 do.	235·6	73·4
After-period:		
-	259·2	55·4
-	233·3	34·4
-	162·3	20·1
-	112·2	20·9

First there is an increase of P excretion, and this is soon followed by a diminished excretion which may fall lower than the excretion during the normal period, though the NH_3 excretion may still be high. Haldane believes this is due to depletion of available phosphates, and when this happens the body tends to produce more ammonia for neutralisation. And during the after-period the excretion of P remains low until it has made up the loss caused by increased excretion during acidosis.

As the subjects both in this experiment and the one quoted before were receiving food during the experiment, and as no figures for the fæcal P are given, the experiments are not conclusive.

Sokhey and Allan (1924) experimenting with dogs to determine the relationship of phosphates to carbohydrate metabolism found that the administration of acid to a fasting dog did not increase the phosphate excretion in urine.

100 c.c. of 0·1N H_2SO_4 were given to a dog weighing 12·0 kg. on the ninth day of the fast. Average phosphate excretion for the fore-period (3rd and 7th day - normal fasting days of the experiment) 71 c.c.0·1M and on the acid day 70 c.c. 0.1M.

(3) Tabular Summary of Experiments quoted.

Author	Experimental Subject.	Acid used.	Fore period.	Acid period.	After period.	Dose of acid per kg per day.	Total Dose per kg.	Diet.	Neutralization by NH_3 1st day.	Maximum neutralization by NH_3 - any day.	Total Excess NH_3 in percentage of acid given.	Increase in fixed base (approx) in percentage of acid given.	Remarks.
			days	days	days	c.c. 0.1N	c.c. 0.1N		%	%	%	%	
Eppinger and Tedesko	Sheep	HCl	4	1	2	250	-	Vegetable diet and plasmon	50	-	93	-	NH_3 never back to normal.
"	"	"	6	1	7/24	107	-	Carbohydrate diet	-	-	14	-	Died.
McCollum and Hoagland	Pig (39)	HCl	27	5	0	59	297	Starch and Salt	20	5th day 36	27	-	Diet changed - no after period
Lamb and Evvard	Pig	H_2SO_4	10	10	8	109	1096	Mixed - Ca rich	17	10th day 100 +	73		
"	"	"	5	10	5	63	630	Mixed - Ca poor	18	10th day 86	73		
Steenbock, Nelson & Hart.	Calf	HCl	4	5	6	34	170	Milk	25	5th day 50	65		
"	"	"	7	24	0	36-74	1410	"	17	7th day 100	86		

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(Continued)

			days	days	days	0.1N cc.	0.1N cc.		%	%	%	%	
Steenbock, Nelson & Hart.	Calf	HCl	9	16	0	22-50	513	Milk		-	42		some urines fermented.
"	"	"	5	7	0	34	170	Straw and Starch	33	-	-		Diet varied.
Gaethgens	Dog	H_2SO_4	4	7	-	42	300	Meat	-	-	-	15	
"	"	"	2	1	2	72	-	Meat extract	38	-	66	17	
"	"	"	3	3	2	41-72	164	Meat & fat	21	-	62	6	
Walter	"	HCl	4	4	2	30-91	272	Meat	37	-	73		
Eppinger	"	"	3	1	4	250	-	Meat	35	-	81		Acid partly subcut.
"	"	"	3	1	4	250	-	Meat & fat	21	-	47		Acid partly subcut.
"	"	"	5	(1)	-	215	-	Starved	-	-	-		Died - acid all subcut.
Secchi	Dog	HCl	7	2	2	45	90	Meat, bread and bone meal	25		(76)		NH_3 never back to normal.
"	"	"	3	3	3	21-64	128	Meat	15		(85)	18	NH_3 never back to normal.
Stehle	"	"	0	12	4	29-58	520	Meat	-	-	(57)	17	Hadn't recovered from glycine hydrochlor.
Keeton	"	"	3	1	3	8	-	Milk, bread Sod. acetate			100	-	
"	"	"	3	1	3	13	-	"			30	-	

(Continued)

			days	days	days	cc. 0.1N	cc. 0.1N		%	%	%	%	
Keeton	Dog	HCl	3	1	3	10	-	Milk, bread Sod. acetate			27	-	
"	"	"	3	3	1	14	-	"			42	-	
Eppinger and Tedesko	"	"	5	1	3	250	-	Meat	51		63	24	
"	"	"	4	(1)	0	150	-	Carbohydrate	(2)		(2)	57	Died after 1 hour.
Dunlop	Man	H_2SO_4	3	2	0	11	22	Mixed	-	2nd day 20	(20)		Diarrhoea no after period.
"	"	HCl	4	3	2	14	41	"	(100)	-	(100)		Cardiac Disease.
"	"	"	3	2	0	10	21	"	50	2nd day 61	(55)	26	Paraplegia no after period.
Coranda	"	"	5	2	3	[11]	[22]	"	38	2nd day 39	80		NH_3 never back to normal.
Limbeck	Man	HCl	3	3	2	7	20	Mixed	0	3rd day 54	38	400	
Camerer	"	"	(1)	2	4	{5-7}	{11}	Variable	-	-	60		Based on after period
Secchi	"	"	3	3	3	9-18	38	Milk & eggs	24	-	72	21	Gastric cancer.
Begun, Hermann & Munzer	"	"	6	10	3	[5-12]	[94]	Mixed	17	10th day 66	61		

(Continued)

			days	days	days	cc. 0.1N	cc. 0.1N		%	%	%	%	
Begun, Hermann & Munzer	Man	HCl	3	3	3	[12-15]	[41]	Mixed	20	3rd day 64	68		
Marriott and Howland	"	"	3	1	1	[7]	-	Ordinary	20	-	20		
"	"	"	3	1	1	"	-	"	24	-	24		
"	"	"	3	1	1	"	-	"	35	-	39		
"	"	"	3	1	1	"	-	"	30	-	41		
"	"	"	2	1	1	"	"	"	23	-	44		)No after period - urine not uniform in fore period.
Stehle and McCarty	Man	HCl	2	3	0	[14]	[43]	Vegetable	17	3rd day 27	21	73	
"	"	"	2	3	0	[14]	[43]	"	10	3rd day 24	17	44	
Zucker	"	"	-	4	0	[4]	[16]	"	-	4th day 100	(59)		Preceded by $NaHCO_3$ Expt.

Foot Note :- [ ] Body weight assumed to be 70 kg.

( ) " " " " " 60 kg.

( ) figures doubtful for various reasons.

PART II.

THE PRESENT WORK.

(1) PLAN AND SCOPE OF THE PRESENT WORK.

The only generally applicable method for measuring the efficiency of the ammonia mechanism consists in * determining the additional ammonia excreted in response to a given dose of acid. The total number of published experiments in which the ammonia excretion has been followed before and after feeding or injecting acid is not far short of a hundred, and a summary of the more important of these has been given, but even among these there are only a few from which it is possible to calculate, with even reasonable accuracy, the relation between the dose of acid and the extra ammonia produced. No generalisation of any sort can be made, based on the entire mass of available data, each experiment being taken at its face value. It has seemed to us, therefore, not only necessary, but perfectly legitimate to impose a number of restrictions. We have left out of account, to begin with, all such cases as are obviously of a special nature: that is, experiments on

(1) pathological human subjects; that is, experiments of Dunlop and Secchi;

(2) growing animals, that is experiments of McCollum and Hoagland, Steenbock and others;

(3) rabbits, that is experiments of Salkowski, Winterberg.

Among the still numerous acid administration experiments on normal, adult animals belonging to species with well-developed ammonia mechanisms, from the greater number it is impossible to determine with any certainty how much of the acid introduced has been neutralised by ammonia, that is experiments of Stehle, Stehle and McCarty, Marriot and Howland, Zucker, Camerer, Limbeck, Coranda, Eppinger and Tedesko. The reason for this is that in some cases the diet was changed in the course of the experiment; in others the after-period was omitted, and occasionally the after-period was too short and the ammonia excretion failed ever to return to its original level - any one of these defects is obviously sufficient to deprive an experiment of any value it might otherwise have for the present purpose. It should be added, of course, that these comments are not necessarily to be regarded as adverse criticism, for some of the work referred to had been planned with a totally different object in view.

Of the comparatively few remaining investigations of this subject, most have dealt with the fate of repeated

doses of acid over periods of from three to ten days. To this group belong the experiments of Gæthgens and of Walter on the dog; of Lamb and Evvard on the pig; of Begun, Hermann and Münzer on man. In the last mentioned case, if the body weight is assumed to have been 70 kg., the daily dose of acid was equivalent on the average to from 9 to 14 c.c. of 0·1N per kg., the total amount administered being, in one of the two experiments done, 40 c.c., and in the other 95 c.c. of 0·1N HCl per kg. In the animal experiments of this group the average daily dose has been between 50 and 90 c.c. and the total between 160 and 880 c.c. of 0·1N acid per kilogram. In spite of such wide variations in the experimental conditions, in all these cases between 60 and 80 per cent. of the acid given is accounted for by excess ammonia excreted in urine. This fair degree of uniformity is in marked contrast to the impression given by an uncritical examination of all experiments of this character that have been published.

As to the fate of single doses much less is known. This is in some respects a special problem, for ordinarily the composition of the urine is altered by the acid during one 24-hour period only - particularly careful control is, therefore, necessary.. Before giving acid, and after its effects have disappeared, the ammonia excretion may, of

course, vary considerably, more or less in proportion to the total nitrogen. In order that allowance may be made for this, as it must be, it is essential that the ammonia co-efficient be practically constant during the fore- and after-periods - otherwise the result can be little more than a guess. Unfortunately, hardly anyone who has worked with single doses of acid has presented experiments properly controlled by total nitrogen determinations, with a satisfactory degree of regularity in the composition of the urine during the control periods, and otherwise suited to the purpose. The only ones, in fact, with any semblance of consistency among themselves are Keeton's four recent experiments on dogs, which were fed between 8 and 14 c.c. of 0·1N hydrochloric acid per kilo in one dose. In one of these, according to the author's calculations, all the acid was neutralized by ammonia; in the other three, ammonia accounted for only 30 or 40 per cent of it. A somewhat lower figure (20 to 30 per cent) represents fairly well the ammonia production on the first day in most of the recorded experiments in which the administration of acid has subsequently been continued over a longer period. Here also the result is the same, as far as we can detect, no matter what the species (always excepting the rabbit). In short experiments, ammonia production seems to be a less important factor, for the average figure which we estimated from the best experiments of

several days' duration was 70 per cent. Two possible explanations for this occur to us; one: that the effect of acid is cumulative; the other that the body has at its disposal a certain, though limited, supply of fixed base which can safely be spared - between the two we are at present unable to decide.

The apparently subordinate rôle played by ammonia on the first day of two of acid administration is of considerable importance in connection with the view, for which we can find no valid evidence at all, that man is inferior to carnivorous animals in his capacity for producing ammonia. This view is very largely based on Camerer and Limbeck's works. Camerer came to the conclusion that very little acid was neutralised by ammonia, but if the after-period of his experiment is used as the control, the extra ammonia comes to about 60% of the acid fed. It is also to be noted that Camerer added potatoes and other vegetables to the diet on the day on which the administration of acid was begun. Limbeck's experiment is quite worthless. He found an increase in fixed base equivalent to about 400 per cent. of the acid; no one, before or since, has reported anything even remotely resembling this. To all the more recent statements in support of the view - since they seem without exception to be based upon comparisons of short experiments on human subjects with the results of prolonged acid feeding to dogs - we cannot subscribe, inasmuch as the duration of the experiment is to all appearances a vital factor.

Except for the occasional, and certainly not typical, instances in which administered acid is quantitatively neutralized by ammonia, there are two other principal mechanisms to be considered in order that the picture may be at all complete. One is the excretion of acid by the kidney (leading to an increase in the titratable acidity of the urine); the other the removal of fixed base from the tissues. The latter is well-known to be the chief means of disposing of acid in rabbits. To what extent it occurs in other animals has been debated at intervals for about fifty years, but so far with no wholly satisfactory answer. Several investigators (Secchi, Limbeck, Eppinger and Tedesko) have been satisfied to determine the combined weight of sodium and potassium in the form of their chlorides; this can have no more than a qualitative significance, as there is no way of expressing the result in terms of equivalents. Before the development of methods for the determination of total fixed base, the desired information could be obtained with accuracy in one way only: i.e., by determining sodium, potassium, calcium and magnesium separately. Few have undertaken this, and of those few none has taken the pains to secure a fore-period showing a fair degree of uniformity in the base excretion for at least a few days, with a return to the same level in the after-period - a very necessary condition, and one which it is not at all difficult to meet, as we have found.

Here again it is only fair to say that many who have been interested in the influence of acid feeding on the excretion of fixed base, although obliged to use quantitative methods to attack the question, have sought only a qualitative answer to it. It is largely because we have tried to get more explicit information than previous writers on the subject that we find their data inadequate. What calculations we have been able to make show, in the great majority of instances, that not far from 20 per cent. of the acid fed has been neutralized by drawing on the body's supply of fixed base.

In what appear to be the most reliable of the experiments upon which this estimate is based, acid was fed for several days in succession. Under similar conditions, as stated earlier, about 70 per cent. of the acid is neutralized by ammonia. There remains, therefore, about 10 per cent. to be covered by acid excretion and any other factors that may be involved - among which may be mentioned especially the neutralization of acid by calcium phosphate in the intestine, and increased phosphate excretion.

If acid were to be fed to a subject on a diet containing a great abundance of phosphate, together with sufficient fixed base to make the urine approximately neutral, there is no doubt that acid excretion could be made to assume a far more important rôle than is ordinarily

the case. It is equally certain that an apparent depletion of fixed base could be brought about by giving acid to a subject on a diet having a strongly alkaline ash. In either case, if carried to extremes, ammonia production would necessarily fall into the background, merely because substitutes for it would be provided in the food. The result, however, could not be accepted as a valid criterion of the body's capacity for producing ammonia. For any real test of the ammonia mechanism, it is essential that the tissues shall not be saturated with fixedbase beforehand, and also that the factor of acid excretion shall as far as possible be suppressed. When these provisions have been met, practically the only alternative to ammonia production is the withdrawal from the tissues of fixed base that constitutes an integral part of them; precisely the occurrence which the ammonia mechanism is designed to prevent. Only under these conditions is it possible to determine how effective that mechanism may be for the work which it is intended to perform.

The foregoing discussion carries with it the implication that fixed base is capable of being stored in the body. To this objections may be raised, but at least in a limited sense there can be no doubt of it, for the concentration of bicarbonate in the body fluids is not a constant but a very variable quantity. When the reaction of the urine is equal to that of the blood, the bicarbonate content of the venous

plasma averages about 0·032 M., distinctly above that which is commonly taken as the normal range; higher levels still would unquestionably be reached on a diet capable of keeping the urine persistently alkaline. Taking the alkali reserve to be uniformly distributed throughout the body fluids - an assumption that is at least roughly in accordance with the facts - we have calculated the amount of fixed base that would be "set free" if the bicarbonate concentration were to fall from 0·032 M to the lower limit of normal. The result is about 70 c.c. of 0·1N base per kilo of body weight, a very considerable quantity in relation to the doses of acid that have ordinarily been used in experiments of the sort now under discussion, especially those done with human subjects. It is hardly conceivable that the result of acid administration could fail to depend to some extent upon whether or not the tissues contain some such quantity of surplus base.

Everyone, as far as we know, who has paid any attention to fixed base excretion in experimental acidosis has found at least some increase. All who have committed themselves to any interpretation at all have implied that there has been a real depletion of base. But if acid administration does no more than rob the tissues of part of a superabundance of fixed base, leaving its concentration within the normal compass, depletion certainly has not occurred.

It is entirely conceivable that the withdrawal of fixed base can be less readily brought about if that event means that the alkali reserve must fall below its normal level. As a corollary to this, the ammonia mechanism may perhaps not come into full play unless there is danger of a real shortage of fixed base.

It is clear, therefore, that the problem is still unsettled in many of its aspects. Being convinced that dietary influences have been a prolific source of confusion in the past, we decided, in going over this ground which had been covered so many times previously, to make one radical departure from the conditions of previous experiments. That is to say, we have used fasting subjects. That many of the uncertainties to which experiments of this nature are liable are thereby at once eliminated will readily be seen.

Besides removing all doubt as to the source of any fixed base that may be appropriated, the fasting basis does away to a very large extent with the disposal of acid by renal excretion - the titratable acidity of the urine in fasting animals is nearly at its limit anyway, and acid feeding cannot have much effect upon it unless the buffer content of the urine is increased, and this under the conditions of our experiments does not occur. By the same device one other complication, inevitable when ordinary food

is present in the digestive tract, is nearly, if not quite, avoided; namely, the accumulation of calcium phosphate and other insoluble basic material, which must necessarily react with acid given by mouth and modify its fate to some extent.

This interaction would also explain the increased phosphate excretion noted by several authors after acid feeding. But all the authors (Steenbock and others, Given and Mendel and Zücker) who made phosphorus determinations both for urine and fæces, found no negative phosphorus balance. Fitz, Alsburg and Henderson's experiments on rabbits, which gave rise to the belief which is still widely held that increased phosphate excretion plays an important role in the elimination of acids from the body must be rejected because: (1) the animals used were rabbits and the results are not generally applicable; (2) acid was given in such large doses and for such prolonged periods that profound secondary effects resulted; (3) the animals were fed but no determinations for phosphorus in fæces were made. For the latter reason the other work quoted from literature is inconclusive.

Residual Fixed Base.

As a matter of convenience, the total fixed base excretion may be considered as made up of two parts. One

of these - often the larger fraction - is represented by base combined as chloride; it is involved, of course, in ordinary salt metabolism, and so is subject to independent variations controlled by the salt intake, the exigencies of osmotic pressure regulation, etc. Of the second fraction a part is combined with weak acids, being distributed among them in a manner which will vary with the hydrogen ion concentration. All, or nearly all, the rest is in combination with sulphuric acid, which, although a strong electrolyte, differs from hydrochloric acid in being almost wholly produced in the tissues from neutral, or at least, less acid substances.

Hydrochloric acid, in other words, occupies a more or less unique position among acids in metabolism. Under ordinary conditions it enters the body fully saturated with base, and is eliminated in the same form. Temporarily, to be sure, it may be separated from its base - in the secretion of gastric juice, as well as in the adjustment of membrane equilibrium in the blood (Zuntz phenomenon) and no doubt elsewhere - and even permanently in connection with the related processes of growth and repair. But if due allowance is made for these special conditions, there are distinct advantages in regarding the fixed base combined with all acids other than hydrochloric as a separate entity.

In following the total fixed base excretion from hour to hour during the post-absorptive period, no definite

simple correlation with any other urinary constituent can be detected. If, on the other hand, the fraction combined with hydrochloric acid is subtracted from the total, a quantity remains which is unmistakably correlated with the ammonia coefficient. To this (i.e. total fixed base minus chloride, both expressed in c.c. of 0·1N) it has seemed best to give the non-committal designation "residual fixed base". The distinction which we have drawn between base combined with hydrochloric acid and the rest becomes, under certain conditions, an artificial one. Thus, if hydrochloric acid is introduced into the body, or if fixed base is withdrawn from circulation for any purpose (e.g., growth) , negative values for the residual fixed base may sometimes be found - meaning, of course, that some hydrochloric acid has been excreted as the ammonium salt. The conception is, nevertheless, useful, and in fact essential, in connection with the present problem. Even in the urine of fasting animals, with its relatively low chloride content, subtracting the chloride equivalent from the total fixed base adds not a little to the information that may be obtained. In the case of (fasting) cats, at least, the ratio of residual fixed base to total nitrogen in the urine becomes fairly constant after the first few days of starvation, whereas the ratio of total fixed base to nitrogen may be distinctly less so, rising and falling with the chloride output when the

latter is irregular. Acid administration may, moreover, affect the salt excretion. If feeding sulphuric acid, for example, should increase the chloride excretion, and to exactly the same degree the excretion of total fixed base - as occurred in one of our experiments - it would be necessary to conclude that no fixedbase hadbeen drawn from the tissues for purposes of neutralization, for the base removed would actually have served no such purpose.

It will be obvious from what has been said that the residual fixed base can have no definite significance in animals which have been fed hydrochloric acid. There is no question, therefore, that the rôle played by fixed base in the neutralization of acid can be more accurately followed if some other acid than hydrochloric is used. This will explain why we have chosen sulphuric acid, which has been practically abandoned in experiments of this nature since the work of Gaethgens in 1880.

(2) EXPERIMENTAL.

Cats were used for all the experiments to be reported. For the reasons given above we had decided to starve the animals right through the experiments, and cats are particularly suitable for starvation experiments. They stand starvation well and no acidosis results from such starvation. Cats very seldom, if ever, urinate in the cage voluntarily and if they do they select the time of the day at which they have become accustomed to having the bladder emptied. This was a particularly important consideration with us, Since ammonia was to be the chief item for analysis, it was important to get perfectly fresh samples of urine for analysis. And to prevent any risk of decomposition even for the few hours during which the urine had to stand between collection and analysis, chloroform was added to the samples, and the samples stored in a cold room.

The cats which were used in the experiments were obtained from the animal room, where they had been fed on meat and skimmed milk ad libitum. They were kept in metabolism cages and starved right through each experiment. To avoid catheterization, the bladder was emptied by compressing the abdomen about three hours before the end of

each 24-hour period, and 100 c.c. of distilled water was given through a catheter used as a stomach tube. At the end of the period the bladder was again emptied in the same manner. The last fraction of the 24-hour urine was in this way made very dilute, and the error, incurred by leaving perchance a small amount of urine in the bladder, was rendered insignificant. In fact, the giving of 100 c.c. of water three hours before the end of the period increases the accuracy of separation into 24-hour samples. The cats were tied to animal boards, specially designed for collection of urine by compression of abdomen, when the urine had to be collected or water to be given.

To the sample obtained at the 21st hour of the period a few drops of chloroform were added and the sample put away in a cold storage room until the second fraction was collected at the end of the 24-hour period. The two samples were mixed and the volume measured.

The ammonia and titratable acidity determinations were made as soon as the 24-hour sample had been collected. The rest of the determinations were carried out as time permitted. The samples were stored in cold storage room until no longer required.

As stated before, the cats were starved for the total duration of each experiment, but were given 100 c.c. of distilled water each day as described above. Fasting had

lasted, in each case, for at least six days before the acid was administered. Within this time the relative composition of the urine had become sufficiently constant, as will be seen from the protocols.

In the experiments which will be discussed first, the acid, (sulphuric) was given per os, by means of a catheter of suitable size used as a stomach tube. The concentration of the sulphuric acid solution was in each instance so adjusted that the requisite quantity could be introduced from a pipette in a volume of exactly 25 c.c., and the catheter was then rinsed out with 5 c.c. of water. To prevent variation in urinary volume, as far as possible, a correspondingly smaller amount of water was given on the acid day at the 21st hour.

For reasons already stated, we have for the present confined our attention to the effect of single doses of acid. Only comparatively small ones have been used in the work comprising this report. One of the questions which we had in mind was whether any increase in fixed base excretion would occur with the smallest doses of acid capable of yielding any decisive result. In choosing the maximum dose, we were guided by the normal daily acid production in fasting cats. 12·5 to 30 c.c. of 0·1N H_2SO_4 per kilogram is the range within which all our present observations lie. This corresponds to the magnitude of the excess acid production in phlorhizinized dogs and cats. We desired not to

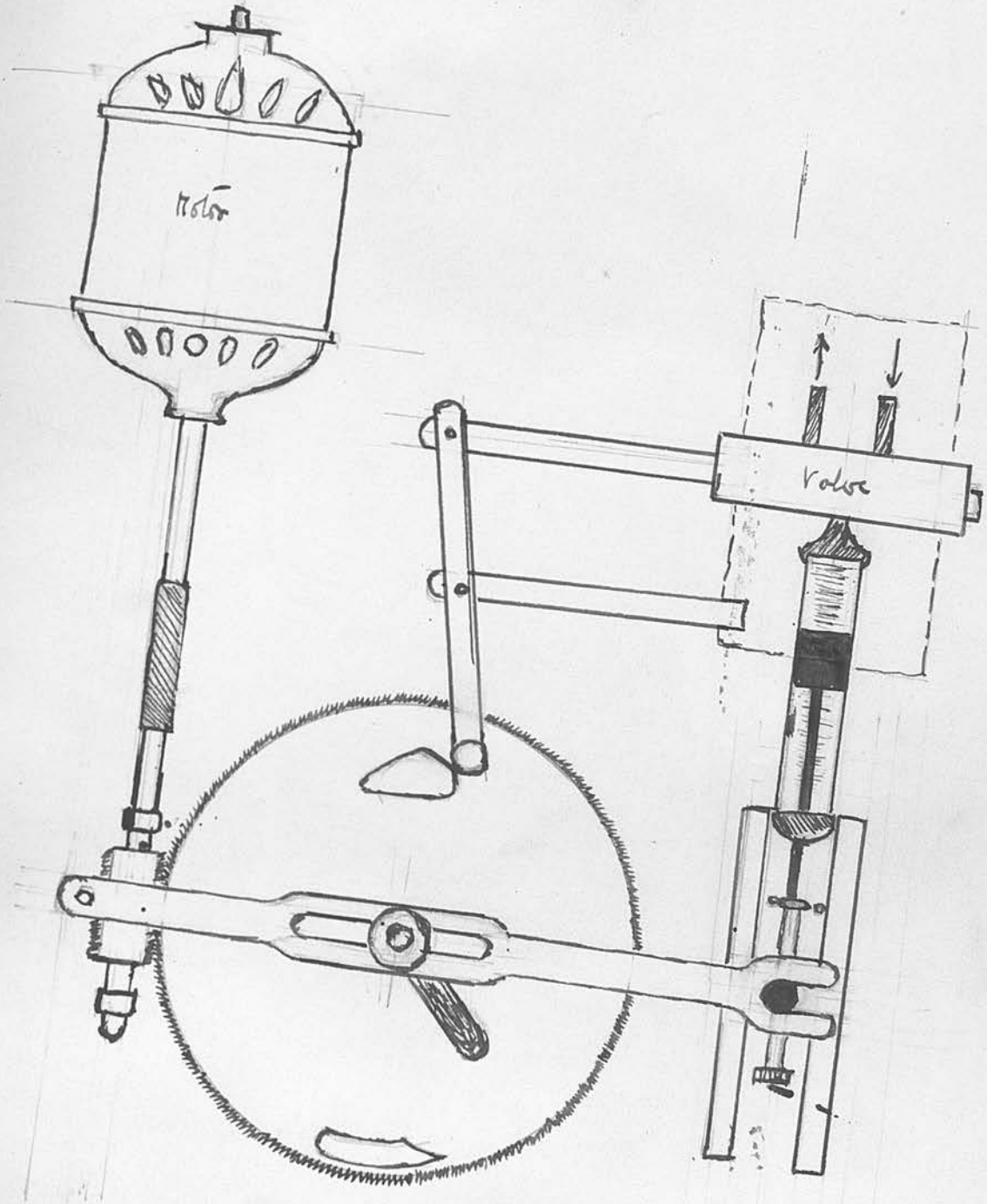
get away from the rate at which acid may be formed in the tissues spontaneously. Our results may be expected to have more bearing on the fate of acid produced under natural conditions than is the case with some previous work, especially that of Eppinger, in which very much larger doses were used.

A still smaller dose (6·25 c.c. of 0·1N per kg.) was also tried on one occasion, but the changes produced in the composition of the urine were too nearly within the limits of experimental error to be of much value.

To ~~some~~ a few animals acid was given by the intravenous route. Sulphuric acid was used for these experiments also. The injections were planned to approximate to the rate of absorption when acid is given by mouth. To get a rough idea of what this might be, the urine excreted in the first 4½ hours after administration of acid in Experiment 4 was collected and analysed separately - about half the acid had by that time been absorbed and excreted, so that much more than half must have entered the circulation. An average rate of 0·05 c.c. of 0·1N acid per kilogram per minute was accordingly chosen for the intravenous injection. But after several unsuccessful attempts to begin the injection at this rate, we eventually discovered that, if still slower rate was used at the start (0·015 c.c. per kilogram per minute), it could be gradually increased at least six or seven times without harm.

In order to secure a uniform rate of injection an automatic injecting machine(illustrated below) was used. The skin over a vein in the leg was shaved and needle inserted into the vein and held in position with strips of sticking plaster.

Subcutaneous injections were given under the skin of the abdomen. Same dosage as given per os was used; samples of urine were collected as described previously.


Motor
Valve

(3) METHODS.

Total Nitrogen was determined by the Folin and Wright's modification of the Kjeldahl method. In this modification, for sulphuric acid of the original method the following acid mixture is substituted. To 50 c.c. of a 5 per cent. copper sulphate solution is added 300 c.c. of 85 per cent. phosphoric acid. To this mixture is added 100 c.c. of concentrated sulphuric acid (free from the least traces of ammonia) and mixed. This mixture gives a very much higher temperature than sulphuric acid.

Details of Method. With a pipette transfer 2 to 3 c.c. of urine to a Kjeldahl flask, capacity 300 c.c.; add 5 c.c. of the phosphoric sulphuric acid mixture and 2 c.c. of 10% ferric chloride solution; add 3 or 4 pebbles, to prevent bumping. Fix the flask in a clamp (in a hood) so that the bottom is only about 1 c.m. above the top of a micro-burner. Boil with a full flame until all the water is driven off and the flask becomes filled with white fumes. At this point cover the mouth of the flask with a watch glass and note the time. Continue the heating (without changing the flame) for two minutes. At the end of two minutes reduce the flame; the white fumes should now be

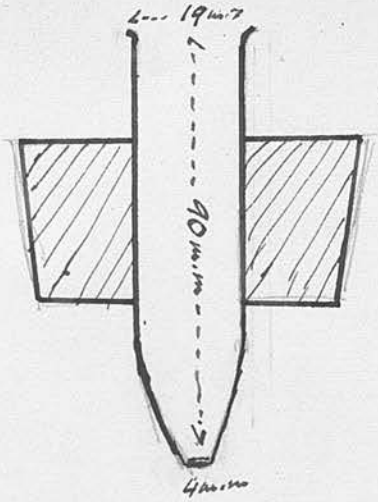
confined within the flask. With the small flame, the heating is continued for two minutes, counting from the time the mouth of the flask was closed. Remove the flame and let cool for not less than four, nor more than five, minutes and then add 50 c.c. of water. Without adding more water to the hot acid solution in the Kjeldahl flask, introduce 15 c.c. of saturated sod. hydroxide and connect promptly into the receiver, which contains from 25 c.c. to 75 c.c. of 0·1N acid, and the total contents of the receiver are made up of the total volume of 150 c.c. with distilled water. With the bottom of the flask only about 1 c.m. from the top of the micro-burner, boil vigorously for five minutes, counting from the time the solution begins to boil hard. At the end of five minutes, withdraw the receiver, allowing it first to rinse itself with steam for a few seconds. Cool the distillate, and titrate.

For calculating the nitrogen from the titration figures, the c.c. of 0·1N acid combined with the ammonia are multiplied by 1·401 and the result gives milligrams of nitrogen.

Sulphate was determined by the method described by Fiske. This method depends on the precipitation of sulphate with benzidine, and its titration with alkali. Benzidine is an extremely weak base, and the benzidine sulphate behaves in this titration as if it were an equivalent amount of sulphuric acid.

Volume of urine containing between 10 and 20 mg. of sulphur as inorganic sulphate is transferred to a 100 c.c. volumetric flask, and diluted to about 50 c.c. with water. One drop of phenolpthalein solution is added, followed by concentrated ammonium hydroxide, drop by drop, until the contents become faintly pink. 10 c.c. of a 5% solution of ammonium chloride are added next, also 1.5 gm. of finely powdered basic magnesium carbonate. Contents are made to the mark, flask stoppered, and shaken for a minute, and allowed to stand for half-an-hour, to allow of the precipitation of phosphates. Contents are then filtered and the filtrate used for sulphur determination.

For inorganic sulphate determination, pipette 5 c.c. of the filtrate into a large lipped Pyrex test-tube. Add 2 drops of a 0·04 per cent. alcoholic solution of bromphenol blue and 5 c.c. of water. Then add N HCl, drop by drop, until the solution is yellow. Run in 2 c.c. of benzidine reagent (suspend 4 gm. of benzidine in about 150 c.c. of water in a 250 c.c. vol. flask. Add 50 c.c. of N HCl, dissolve, and dilute to the mark), and let stand for two minutes. Finally, add 4 c.c. of 95 per cent. acetone free of aldehyde, and let stand for ten minutes. Meanwhile, prepare a thin mat of paper pulp in the bottom of the special filtration tube of the form illustrated in the diagram.



On this mat, filter the benzidine sulphate precipitate with very gentle suction. Wash by rinsing down the sides of the Pyrex test tube with 1 c.c. of 95% acetone and transferring to the filtration tube; wash twice more with 1c.c. of acetone, and once with 5 c.c.

With the aid of a little water, poke the precipitate and mat through the hole in the bottom of the filtration tube back into the Pyrex test tube. Rinse off the wire with a few drops of water, and titrate the contents of the test tube (hot) with 0·02 N NaOH, after adding 2 drops of 0·05 per cent aqueous solution of phenol red. In order to be sure that all the precipitate is removed from the filtration tube, the latter should be left suspended in the mouth of the test tube until about 1 c.c. of alkali has been run in through the filtration tube. The tube is then rinsed with 2 or 3 c.c. of water from a wash bottle, and further washed by boiling the solution until steam escapes from the mouth. After another rinsing with a few c.c. of water the filtration tube may be removed and the filtration

completed, until 0·02 c.c. of alkali produces a definite pink color that remains on boiling again.

The amount of inorganic sulphur in the 5 c.c. of the filtrate analysed is obtained (in mg.) by multiplying the titration figure by 0.32.

Total Sulphate. To 5 c.c. of the same filtrate in a 100 c.c. beaker, add 1 c.c. of 3 N HCl. Heat on a water bath until the solution has evaporated to dryness and for 10 minutes longer. Transfer the contents of the beaker to a lipped Pyrex test tube with five 2 c.c. portions of water, add 2 c.c. of benzidine reagent and proceed as for inorganic sulphate.

The author in preliminary experiments with this method obtained results showing an error not greater than one per cent. with known solutions.

Total Fixed Base was determined by the method described by Fiske. It consists in the conversion of fixed base into sulphate, and the precipitation of the sulphate so formed, with benzidine, and titration with alkali of the precipitate suspended in water as described above.

Measure into a large-lipped Pyrex test tube (200 x 20 m.m.) a sample of urine representing about 0·1 hour. (The sample should not contain more than 5 mg. of inorganic phosphorus, so less than the amount indicated must be used if the phosphorus content is more than 50 mg. per hour or

1·2 gm. per day.) Add 1 c.c. of approximately 4 N Sulphuric acid, 0·5 c.c. of conc. nitric acid, and a quartz pebble, and boil down until white fumes appear. If the residue does not soon become colourless after this stage has been reached, cool slightly, add a few drops more nitric acid and continue the heating. When the remaining drop of sulphuric acid has become clear and colourless, let cool for a few minutes and rinse into a test tube (accurately marked at 25 c.c.) with four portions of 2 c.c. portions of water. Add a drop of saturated alcoholic solution of methyl red. Neutralise with powdered ammonium carbonate until the colour of the indicator just begins to change, and restore the pink color by adding 4 N sulphuric acid, one drop at a time. Heat to boiling and again restore the pink colour, if necessary. Add a 10·5 per cent solution of ferric chloride crystals in 0·1N HCl in the proportion of 0·1 c.c. for each mg. of inorganic phosphorus present, shake, and run in 1 c.c. of a 5% solution of ammonium acetate. Add sufficient water to make the total volume 10 or 11 c.c., heat again to boiling, and dilute to 25 c.c. mark with cold water. Close the mouth of the tube with a clean, dry rubber stopper, invert 2 or 3 times, and filter immediately through a dry 9 c.m. ashless paper into a dry test tube. The filter should be kept nearly filled as long as possible, and only about 20 c.c. of filtrate should be collected. Stopper the tube containing filtrate and cool.

Transfer 5 c.c. of the filtrate to a small platinum dish, add 1 c.c. of 4 N H_2SO_4, and evaporate on a water bath until dry. Place the dish on a metal triangle and heat, cautiously at first, gradually rising flame until fumes have ceased to come off. Let cool, sprinkle over the residue a little powdered ammonium carbonate and ignite again, finally raising the flame to its maximum and moving the triangle about until each part of the dish has been momentarily subjected to a dull red heat. When the dish has been cooled, add 2 c.c. of water. Agitate until the residue is dissolved using a rubber-tipped rod to assist in dissolving it if necessary. Transfer the contents of the dish to a large piped Pyrex test tube and rinse four times with 2 c.c. of water. Determine the sulphate content of the solution by the benzidine method described above.

If 0·02 N NaOH is used in titrating the benzidine sulphate precipitate, the titration figure (after subtracting a temperature correction of 1 per cent.) gives the number of of c.c. of 0·1N fixed base in the sample of urine.

The order of accuracy of this method is that of the sulphate determination method.

Inorganic Phosphate. was determined by Brigg's modification of Bell and Doisy's colorimetric method.

This method depends on the reduction of phosphomolybdic acid with the formation of a blue colour. If molybdic acid is added to a solution of phosphoric acid (i.e. an acidified solution of a phosphate, phosphomolybdic acid is formed. If the solution is reduced with hydroquinone a blue colour is formed, the intensity of which is proportional to the amount of phosphate originally present. Molybdates are not reduced by hydroquinone. The addition of sod. sulphite deepens the blue colour.

The method is applied as follows: Transfer one to five c.c. of urine, according to concentration of phosphate, into a 100 c.c. flask; transfer 5 c.c. of standard monopotassium phosphate solution containing 0·5 mg. of phosphorus into another similar flask. To each flask add about 25 c.c. of phosphate free distilled water, 5 c.c. of molybdic acid solution (this is a 5% solution of ammonium molybdate in normal sulphuric acid) 1 c.c. of ·5% Hydroquinone solution and 1 c.c. of 20% Sod. sulphite solution. Solutions are gently mixed and allowed to stand for 30 minutes and made to the mark, and the color of the standard solution is compared with that of the urine, setting the standard at 20 m.m. reading of the colorimeter.

20 divided by the reading for urine, multiplied by the phosphorus in the standard gives the phosphorus in urine taken.

This method gave results with an error of less than 1·5%.

Ammonia was determined by the aeration method of Folin and McCollum. This method depends on the liberation of free ammonia on the passage of strong current of air through urine made strongly alkaline. The ammonia thus set free is received in dilute acid; it is Nesslerized and the depth of the colour resulting is compared with the depth of colour of a standard solution of Ammonium Sulphate similarly Nesslerized.

Details of the method are: With an Ostwald pipette measure 2 or 3 c.c. of urine into a large Jena test tube (200 x 25 m.m.). Choose the amount which contains nearer 1 mg. of Nitrogen. Fit the test tube with a two-hole rubber stopper carrying an inlet tube, reaching to the bottom, and an outlet tube. Connect the former with the compressed air jet, and the latter with an absorption tube having small holes drilled through the wall at the end. Insert the absorption tube into a 100 c.c. vol. flask containing 20 to 30 c.c. distilled water and 2 c.c. 0·1N HCl. Add 2 drops kerosine and a few drops of a solution containing potassium oxalate and potassium carbonate (15% of each), quickly put the stopper firmly into the tube and start the air current, gradually increasing its speed for about 2 minutes. In ten minutes all the ammonia should be driven over into the receiving flask.

Remove the absorption tube, rinse with water and dilute contents in the flask to about 75 c.c. Pipette10 c.c. of of standard ammonium sulphate (containing 1 mg. nitrogen) into

another 100 c.c. vol. flask and dilute with water to 60 c.c.

Nesslerize both solution and make colour comparison with colorimeter.

When the current of air has been standardised by preliminary experiments, aeration for ten minutes gives results with an error of less than 1%.

Chloride Determination gave considerable amount of trouble. Direct titration could not be carried out. It was found that the addition of silver nitrate and nitric acid to the urine of cats, even during fasting, caused the appearance of a dark coloured precipitate, indicative of the presence of sulphur compounds of the nature of thiosulphate. Removal of this interfering material became essential.

We first employed a modified Volhard method after destroying the organic matter by fusion with alkali.

Details of the method as employed are:-

Five c.c. of urine were taken in a platinum dish and to this was added 1 c.c. of a saturated solution of sodium carbonate and 1 c.c. of a saturated solution of Potassium chlorate. The mixture was evaporated to dryness on a water bath. The dried material was then ignited. The ash was dissolved in 2 c.c. of water and transferred to a large-sized centrifuge tube, and the dish rinsed four times with

2 c.c. portions of water and the washings added to the centrifuge tube.

To this was added 1 c.c. (or as required) of 0·05 N $AgNO_3$ solution, a few drops of HNO_3 and a few drops of ferric alum solution, as an indicator. The excess of silver nitrate was titrated with a 0·01 N.solution of KCNS. KCNS was added until the first signs of paramount reddish colour appeared. The solution was then centrifuged and the precipitate AgCl and AgCNS thrown down, and the titration finished on the supernatant liquid.

The titration figure subtracted from 5 c.c. (as $AgNO_3$ solution is 0·05 N) and the result divided by 10 gives the c.c. of 0·1N Cl solution in the urine taken.

As ammonia determinations formed so important a part of the investigation, it was desired to run no risk of decomposition even for the few hours during which the urine had to stand between collection and analysis, and preservation with chloroform in the cold room was adopted as the means least likely to interfere with any of the analytical operations. We relied upon statements in the literature, particularly Halverson and Schulz, that chloroform was without effect on this method. After the chloride determinations for two experiments had been carried out, we found that chloroform did interfere with this method of determination, and in consequence we have no reliable figures for chloride in the first two experiments.

For the rest of the work the ashing was accomplished by boiling with nitric acid and permanganate, as suggested by Koranyi.

Details of this method are as follows: 5 c.c. of urine were transferred to a large Pyrex test tube; to this a few drops of nitric acid were added and the urine brought to a boil. Now saturated potassium permanganate solution was added drop by drop until the urine became colourless; boiling was continued while permanganate was being added. Urine was now allowed to cool, and when cold 1 c.c. (or as required) of 0·05 N $AgNO_3$ solution was added and titration with KCNS completed as described above.

(4) RESULTS AND DISCUSSION.

1. Sulphuric Acid per Os.

Analyses of the urine from our oral administration experiments are presented in Tables II to IV, while in Table V will be found the results in summarized form, showing to what extent ammonia and fixed base have participated in the neutralization. From the 4th column of Table V. it will be seen that we have in most instances failed to recover all the sulphuric acid fed; usually only about 80 per cent. appears in the urine as inorganic sulphate. Others who have worked with sulphuric acid have had a similar experience. While the precipitation of calcium sulphate in the intestine, which has been suggested as an explanation, is less likely to occur in fasting animals, we cannot say that it is impossible, for the large intestine after a week's starvation contains an abundance of fæcal material. In one case in which this was collected and analyzed, 27 mg. of calcium were found to be present.

Whether the sulphuric acid is incompletely absorbed, or whether the absorption of the last fraction of it is merely delayed, failure to reach the circulation promptly is almost certainly the reason for our inability to find it all in the urine - no such discrepancy has occurred in our experience

TABLE I.

Base-Nitrogen Ratios.

Method of administration.	Per os.							Subcutaneous.		Intravenous.	
Dose of Acid, cc. 0.1 N per kilo.	30		25			12.5		25		12.5	
Experiment No.	2	1	3	10	4	6	15	16	8	11	
Day of fast.	Ammonia (cc.0.1N) per gm. nitrogen.										

TABLE I (Contd.)

1	44		27						
2	41		28						
3	34	45	25		21				
4	28	32	27	47	23	26	50	33	26
5	25	32	28	40	23	25	47	32	26
6	24	26	23	34	27	26	50	28	28
7	50†	44†	50†	33	39†	40†	69†	31†	14†
8	26	29	28	54†	30	25	51	19	7
9	22	26	26	40		27			
10	[illegible]	28		43	24				
11				43	24				
12					34†				
13					22				

Residual fixed base (cc.0.1 N) per gm. nitrogen.

1			20						
2			26						
3			42		36				
4			35	22	34	28	34	23	24
5			35	29		29	32	34	31
6			39	36	33	30	35	35	36
7			62†	34	36†	54†	65†	54†	176†
8			32	38†	30	30	34	35	81
9			35	35		30			
10				29	32				
11					34				
12					48†				
13					37				

† Day of acid administration.

TABLE II.

Sulphuric Acid per Os (30 c.c.0.1 N per kilo).

Experiment 2. Fasting Cat. 2·05 kilos.

Day of fast	Volume	Total N.	Inorganic P.	Inorganic Sulphate	Ammonia	Total fixed base	Remarks
	c.c.	gm.	c.c.0.1M	c.c.0.1N	c.c.0.1N	c.c.0.1N	
2	101	1.74	42	45	71	54	
3	120	1.77	45	47	61	63	
4	102	1.82	46	47	50	72	
5	105	1.85	43	47	47	69	
6	103	1.97	48	52	47	84	
7	120	2·08	46	105	104	115	25 c.c.0.245 NH_2SO_4 (equivalent to 61·3c.c. 0.1 N.)
8	117	1·71	45	48	44	74	

TABLE III.

Sulphuric Acid per Os (25 Cc. 0.1 N per Kilo.

Day of fast.	Volume.	Total N.	Inorganic P.	Inorganic sulphate.	Ammonia.	Total fixed base.	Residual fixed base.	Cl	Remarks
Experiment 1. Fasting cat. 2.11 kilos.									

TABLE III (Contd.)

	cc.	gm.	cc. 0.1M	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1N	
3	85	1.45	40	38	65	62			
4	97	1.67	40	46	54	68			
5	98	1.54	37	43	50	60			
6	101	1.51	38	38	39	62			
7	107	1.69	43	86	75	93			0.25 cc.Ø. 0.211 N H_2SO_4 (equivalent to 52.8 cc. 0.1N)
8	105	1.85	44	50	53	70			
9	81	1.37	37	38	36	69			
10	97	1.55	40	46	44	65			
				Experiment 3. Fasting cat. 2.55 kilos.					
2	112	1.74	33	32	49	57	45	11.7	
3	105	1.44	33	30	36	64	60	13.8	
4	92	1.23	25	24	33	50	43	6.8	
5	95	1.20	32	23	33	47	42	5.1	
6	105	1.13	27	24	26	48	44	4.4	
7	115	1.13	27	83	57	74	70	3.6	0.25 cc.Ø. 0.255 N H_2SO_4 (equivalent to 63.8 cc. 0.1N)
8	80	1.05	26	27	29	36	34	2.1	
9	87	1.02	23	21	27	39	36	2.9	
				Experiment 10. Fasting cat. 1.12 kilos.					
4	94	0.86	20	17	41	25	19	6.0	
5	122	0.94	22	19	38	34	27	7.0	
6	107	1.07	22	24	36	56	38	17.5	
7	107	1.05	20	24	35	52	36	16.1	
8	95	0.82	19	40	44	38	31	7.0	0.25 cc.Ø. 0.112 N H_2SO_4 (equivalent to 28.0 cc. 0.1N)
9	96	0.82	19	22	33	36	29	7.4	
10	98	0.80	21	21	34	31	23	7.8	

TABLE IV

Sulphuric Acid per Os (12.5 Cc. 0.1 N per Kilo).

Day of fast.	Volume.	Total N.	Inorganic P.	Inorganic sulphate.	Ammonia.	Residual fixed base.	Cl	Remarks.
	Experiment 4. Fasting cat. 2.35 kilos.							
	cc.	gm.	cc. 0.1M	cc. 0.1 N	cc. 0.1N	cc. 0.1N	cc. 0.1N	
10	102	1.11	23	23	27	39	2.9	
11	92	1.06	28	22	25	36†		25 cc.0.118 N H_2SO_4 (equivalent to 29.5 cc. 0.1N).
12	113	1.01	24	52	34	54	1.8	
13	96	1.05	25	23	23	39†		
	Experiment 6. Fasting cat. 2.75 kilos.							
3	115	1.17	29	21	25	42	5.9	
4	125	1.11	25	20	26	38	7.5	
5	85	1.00	20	19	23			25 cc.0.138 N H_2SO_4 (equivalent to 34.5 cc. 0.1N).
6	83	0.92	21	18	25	30	5.9	
7	103	1.09	20	49	42	39	5.1	
8	94	0.94	22	17	28	28	6.6	

† Owing to shortage of material, the chloride in these two samples was determined only by the fusion method. The figures for residual fixed bases are therefore too low, to the extent of 2 cc. of 0.1 N or less.

TABLE V.

Neutralization of Sulphuric Acid by Ammonia and Fixed Base.

Dose of acid per kilo.	Method of administration.	Experiment No.	Inorganic sulphate recovered.	Neutralized by		
				Ammonia.	Residual fixed base.	Sum.
cc. 0.1 N			per cent	per cent	per cent	per cent
30	Per Os.	2	82	104	(0)‡	(104)
25	"	1	83	64	(46)‡	(110)
25	"	3	88	51	55	106
25	"	10	75	67	15	82
12.5	"	4	103	32	42	74
12.5	"	6	77	60	18	78
25	Subcutaneous.	15	100	34	56	90
25	"	16	100	47	70	117

‡ Approximate figures, based on chloride determinations made by the fusion method (see text).

when sulphuric acid has been injected parenterally. The sulphate recovered from the urine has, therefore, been assumed to be identical with the amount of sulphuric acid absorbed, and upon this, rather than the total dose, we have based our calculations (Table V).

The method by which we have computed, from the urine analyses, how much extra ammonia and fixed base have appeared in the urine will need some explanation, since it differs from those ordinarily used. In fasting animals, the chief source of the urinary constituents with which we are concerned here excepting, of course, the period during which the animal is under the influence of acid given - is muscle. As is well known from observations on other animals, the amount of muscle catabolized during starvation may vary considerably from day to day; and cats, as a rather extensive experience has taught us, are no exception. The total nitrogen excretion, in short fasts at least, is nearly always either rising or falling progressively - and ammonia, sulphate, phosphate and residual fixed base, to mention only the things which now particularly concern us, follow it in whichever direction it may be tending. After the first few days of starvation they all - barring exceptional cases - become and remain proportional to the total nitrogen, within 10 per cent or less.

That variations in the level of protein metabolism should be taken into account in calculating ammonia production is quite

beyond dispute, although usually this has not been done in the past. The method of calculation is identical in principle with the familiar use of the G:N ratio in experiments on phlorrhizinized animals. The ratios required in the present case - ammonia and residual fixed base respectively, to total nitrogen - have been collected into a separate table (Table V.), for it is only by the examination of them that the reliability of the calculations may be judged. In Experiment 1, for example, the ammonia ratio (c.c. of 0·1N ammonia per gm. of nitrogen) was 26 on the day before, and 29 on the day following that on which acid was fed. The average of these two figures (27·5), we assume, is what the ratio would have been on the experimental day if no acid had been given; subtracting it from the ratio actually found (44) leaves 16·5 c.c. of extra ammonia produced, per gm. of total nitrogen excreted, in response to the sulphuric acid administered. The total nitrogen on that day (Table II.) was 1·69 gm., therefore the total extra ammonia excreted was 1·69 x 16·5, or 28 c.c. of 0·1N. Of the acid given, 44 c.c. of 0·1 N were recovered from the urine, as determined by a similar calculation, so the fraction neutralized by ammonia was 28/44, or 64 per cent.

All the other results in Table V. were computed by the same method. They show, first of all, that on the average about 60 per cent. of the sulphuric acid given by mouth and subsequently recovered from the urine has been neutralized by ammonia. If

based upon the entire dose of acid, the average would come to about 50 per cent, a distinctly higher figure than the general run of results that have been reported with animals which were fed during experiments (P. 60). In this there is some support for the view, expressed in the introductory part of this paper, that a certain amount of acid may be neutralized by basic constituents of the food which have accumulated in the tissues. There is certainly no merit in the contention of Eppinger that starving animals are incapable of producing ammonia to protect themselves against administered acid. Eppinger's contention was that tissue protein cannot serve as a source of ammonia. As far as Carnivora are concerned, his only basis for this view was one improperly controlled experiment on a dog, intended to show that the lethal dose of acid is less during starvation; no analyses were made, for the dog did not survive. Either Eppinger believed that acid introduced into the body from without is a special case, or else he overlooked a number of well-known facts. Ammonia production for the neutralization of acid metabolic products, formed within the body, is of course incontestable - the high ammonia excretion in fasting man (with acidosis) is all the proof that need be cited.

In one experiment(No.2) apparently the entire burden of neutralization fell on the ammonia mechanism. Occasional results not unlike this may be found scattered through the literature, but they are not at all typical.

It was in the first two experiments of the series that the chloride determinations were done by the fusion method, with results that are distinctly too high. In these two cases we are, therefore, unable to give accurate figures for the residual fixed base. The total fixed base was, nevertheless, determined, and in Experiment 2 it was found to have risen markedly on the day of acid administration. From that fact it might be inferred that a considerable part of the acid had been neutralized by fixed base, and that the total extra base eliminated (including ammonia) was far more than the equivalent of the sulphuric acid absorbed. Fortunately, the two chloride methods - fusion and the permanganate method which we finally adopted- were compared on more than 30 samples of urine, all of which were preserved with chloroform. While the error in the former method is considerable, it is always about the same, and for comparative purposes the results are not entirely without value. On the day in question, the chloride output as a matter of fact rose also, and to practically the same extent as the total fixed base - by the nearest estimate that we are able to make, there was no increase in the residual fixed base at all. This is perhaps the most striking evidence that we can give to show the importance, really amounting to indispensability, of chloride determinations in connection with the present problem. It follows with equal force that hydrochloric acid is not a suitable substance to use in such work.

In Experiment 1, as nearly as we can tell - since the same inaccurate chloride method was used here also - the increase in fixed base was fully enough to cover the fraction of acid not neutralized by ammonia.

One of the objects in mind in choosing fasting animals as subjects was, as we have said, to exclude the possibility of neutralization by fixed base derived from the food and temporarily stored in the tissues. The fixed base is, nevertheless, increased in nearly every case after the feeding of sulphuric acid to fasting cats, but the quantity varies greatly. In Experiment 2, for reasons already given, none could be detected within the experimental error, in this case comparatively large. In Experiments 6 and 10 the amount is small - equivalent to 18 and 15 per cent respectively of the sulphate - but apparently well outside the limit of error. In the other three experiments (1, 3 and 4) about half the sulphuric acid was neutralized by fixed base. The only explanation which we can see for these variable findings is that the amount of fixed base available for the neutralization of acid is not the same in different subjects. From this it seems to follow that - at any rate with the comparatively small doses which we have used- if acid feeding deprives the body of fixed base at all, it draws only on a supply which is not particularly needed, and which is therefore not tenaciously retained.

From one standpoint the amount of fixed base lost, even in the most extreme instance, is small. 55 c.c. of 0·1N base, in a cat of 2·55 kilos (Experiment 3), is only 22 c.c. per kilo body weight. If the "alkali reserve" may be assumed to be uniformly distributed throughout the body fluids, a loss of this magnitude would diminish the concentration of base by only 0·003 N., equivalent to 6·7 volumes per cent. carbon dioxide - a small fraction of the normal range of variation in the bi-carbonate content of plasma.

We believe, therefore, that we have not succeeded, with 30 c.c. of 0·1N acid or less per kilo, in withdrawing from the tissues fixed base that cannot well be spared.

As noted earlier, there is some uncertainty about the calculation of extra ammonia excretion in Experiment 10, because the ammonia coefficient rose slightly but permanently during the last few days - probably the figure given for neutralization by ammonia is underestimated. Only with the smallest dose tried (12·5 c.c. per kilo) do the ammonia and residual fixed base together definitely fall short of covering all the sulphuric acid absorbed. (Experiments 4 and 6). Presumably the rest has been disposed of by the process of acid excretion, but the most that we can say on this point is that a sufficient increase in titratable acidity to account for what remains may have occurred. Acidity titrations were done in

nearly all these experiments, but not inconsiderable variations were found in the control periods, and any slight changes due to acid feeding cannot readily be detected. Evidently, by the use of fasting subjects, we have eliminated acid excretion as a factor - as indeed we had planned to do - not entirely, but so nearly that no accurate estimate of its magnitude can be made.

2. Parenteral Administration of Sulphuric Acid.

a. Intravenous Injections.

As quoted above, Keeton (1921) had noticed from literature that ammonia mechanism did not come into play equally effectively in all forms of acidosis. He divided the cases of acidosis reported into two categories: (1) those in which ammonia excretion reached a high level, and (2) those in which ammonia excretion did not seem to be an important factor. In the first category the acid was always hepatogenous in origin, or was introduced into the body by the alimentary route and very largely passed through the liver after absorption. He enunciated the hypothesis, that ammonia is produced to neutralise acid only if the acid either arises in the liver or enters the liver through the portal circulation. He undertook to verify this hypothesis by experimental work. He gave acid by the mouth and by the intravenous route, and found that when the acid was administered per os, a considerable fraction of it proved to have been neutralised by ammonia, while the same dose when given intravenously produced little or no increase in the ammonia excretion. He therefore

TABLE VI.

Sulphuric Acid Intravenously (12.5 cc.0.1 N per Kilo).

Day of fast.	Volume.	Total N	Inorganic P.	Inorganic sulphate.	Ammonia.	Residual fixed base.	Chloride.	Remarks.
Experiment 8. Fasting cat.2.14 kilos.Injection started very slowly.no hype								
	cc.	gm.	C.C.O.IM	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	
4	87	1.07	25	22	35	25	4.5	
5	106	1.09	27	24	35	37	6.1	
6	88	0.90	24	19	25	31	7.8	
7	129	1.26†	21	46	39	68	18.4	26.7cc.0.1N H_2SO_4 injected in 3½ hours
8	90	1.26	27	23	24	44	2.5	
Experiment 11. Fasting cat. 2.58 kilos. Air hunger.								
5	100	1.33	33	24	35	41	11.9	
6	63	1.26	33	23	35	45	4.2	
7	82	0.70‡	35	55	10	123	35.1	32 cc.0.1N H_2SO_4 injected in 4 hours.
8§	110	0.90‡	18	31	6	73	57.0	

† Non-protein nitrogen. The protein nitrogen was 52 mg.

‡ " " " " " " 79 mg., including 5 mg in the form of haemoglobin. pH 7.4.

‡ Non-protein nitrogen. " " " " 20 mg.

§ 21 hour urine. The cat died before the end of the period.

surmised that the liver was ~~the seat of~~ intimately related to ammonia formation. This contradicted the work of Nash and Benedict whose work tended to show that the kidney was the chief seat of ammonia production.

Bayliss (1919), before Keeton, had reported one experiment on intravenous administration of acid to a dog.. He determined the ammonia in the urine secreted before the injection was begun and for a period lasting up to 77 minutes after it had been completed. He observed no significant increase in ammonia production. He drew the conclusion that ammonia production does not appreciably come into play in short experiments. Bayliss thereby laid stress on the time element, while Keeton emphasised the route of administration. Bayliss made no attempt to control his experiments by using other paths of administration as was done by Keeton.

Hoping to be able to throw some light on this rather curious situation we undertook a number of experiments of intravenous injection of sulphuric acid. The task proved to be far more difficult than we had anticipated. We noticed that acid injection into a cat's vein, no matter how slowly done, always resulted in haemoglobinuria. Bayliss had noticed the same difficulty. We found that though the actual amount of haemoglobin excreted was small, it was always accompanied by a much larger quantity of

albumin, showing that the kidney was invariably damaged, and the normal proportion between total nitrogen and other waste products disturbed. We were, in consequence, unable to rely upon the method of calculation by means of which we had followed the ratio of ammonia produced to the acid given in the experiments of acid per os. Our results, however, show: (1) that there is little or no increase in ammonia, and (2) that a sufficient rise in residual fixed base excretion occurs to account for all the acid injected and sometimes even more than that. The first of these observations confirmed Keeton's work; the second furnished a clue to their interpretation.

Judging by the facility with which haemolysis is produced, cats are unusually susceptible to acid given intravenously, for other species that have been tried rarely show any effect. Szili in his experiments on various species noticed haemolysis in a few sheep. Oré gave acid intravenously to dogs and does not mention whether he noticed any change in the urine. Guttman used rabbits in repeating Oré's work. He also did not notice haemoglobinuria.

Further evidence of abnormal behaviour of cats we encountered in distressing abundance, for all our first attempts terminated fatally. We shall not describe all the efforts that we made to surmount our difficulties, but we did finally find a way out.

The injections were planned to approximate the rate of absorption when acid is given by mouth. To get a rough idea of what this rate might be, the urine secreted in the first 4½ hours after administration of acid in Experiment 4 had been collected and analysed separately. We found that about half the acid had by that time been absorbed and excreted, so that much more than half must have entered the circulation. An average of 0·05 c.c. 0·1N acid per kilogram per minute was accordingly chosen for the intravenous injections.

After several unsuccessful attempts to begin the injection at this rate, we eventually discovered that if still slower rate was used at the start (0·015 c.c. 0·1N per kg. per minute), it could be gradually increased at least 5 or 6 times without harm. An automatic injecting machine had to be used for giving injections so slowly. This was the device adopted in Experiment 8 (Table VI.) where we were not troubled even with hyperpnoea which Bayliss was unable to avoid except by the use of morphine. The respiratory rate remained below 40 per minute with the exception of a very short period early in the experiment, when it rose to 48.

By our usual method of calculation we find that 15 per cent. of the sulphuric acid was in this case neutralised by ammonia, and 90% by fixed base. Whether the ammonia production is real or only apparent, we can

see no way of deciding, but at all events this particular experiment is not seriously inconsistent with Keeton's observations. The next one, however, gave very different results.

When no special precautions were taken to avoid hyperpnoea, the results were very different. In Experiment 11 (Table VI.) we tried to control the respiration by making the injection intermittent, the rate varying between 0·04 and 0·07 c.c. of 0·1N per kg. per minute. But we did not succeed. Within the first 20 minutes pronounced air hunger appeared. Although the injection was then entirely suspended for nearly an hour, the respiratory rate remained well above 100 for some time, and was still about 80 (with no sign of falling further) when the injection was resumed. Nevertheless, during the last 140 minutes of the injection, when 75 per cent. of the whole dose was introduced at the rate of 0·07 c.c. per kg. per minute, the respiratory rate was only 45 - 57.

The analytical results of this experiment are very instructive. It is perfectly clear that, in one sense, the acidosis was more than compensated for by over-ventilation, for the extra residual base excreted was about 2·5 times the equivalent of the acid injected. With all this fixed base liberated, it is no wonder that the ammonia excretion actually fell, or that the urine

became alkaline. We can not escape the suspicion that the same compensatory process, in milder form, is the real cause of apparent absence of ammonia production when no very serious respiratory disturbance takes place. Respiratory disturbance in our experiment is extreme, but a disturbance less marked might easily remain unnoticed.

b. Subcutaneous Injection.

Having been thus led to doubt the soundness of Keeton's explanation - namely, that acid entering the systemic circulation directly is not excreted combined with ammonia at all - we were able to settle the question under less severe conditions. Very slow intravenous injections over very prolonged periods were tried unsuccessfully, for in cats the vein is inclined to get clogged by agglutinated corpuscles. For the main point at issue, the precise rate of entrance to the circulation is of no particular importance, and subcutaneous injections serve the purpose quite as well.

Acid given subcutaneously in doses of 25 c.c. of 0.1N per kilogram (Table VII and V.) is neutralised by ammonia to the extent of about 40 per cent. No great significance can be attached to the fact that less ammonia is formed than when an equal quantity of acid is given by the mouth. With a larger number of experiments the difference might conceivably disappear. Furthermore, the absorption of acid is no doubt more rapid from the subcutan-

TABLE VII.

Sulphuric Acid Subcutaneously (25 Cc.0.1 N per Kilo).

Day of fast.	Volume.	Total N.	Inorganic P.	Inorganic sulphate.	Ammonia.	Residual fixed base.	Chloride.	Remarks.
			Experiment 15. Fasting cat. 2.56 kilos.					
	cc.	gm.	cc. 0.1M	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	
4	78	1.49	30	20	39	41	5.4	
5	90	1.50	30	20	38	44	5.0	
6	103	1.49	33	18	39	45	5.4	
7	127	1.52	31	86	61	82	4.3	25 cc. 0.1N H_2SO_4
8	98	1.54	30	27	38	46	12.8	
			Experiment 16. Fasting cat. 1.59 kilos.					
4	85	0.97	23	25	48	33	2.3	
5	80	0.96	22	26	45	31	3.6	
6	90	0.91	22	25	45	32	3.4	
7	89	0.93	24	65	64	60	2.5	25 cc. 0.1N H_2SO_4
8	85	0.85	22	23	43	29	6.8	

eous site, and we can not be sure that the causes of the peculiar results of intravenous injection are wholly absent here. The formation of ammonia to neutralise acid entering the peripheral circulation is at all events unquestionable.

3. The Influence of Acid on Phosphate Excretion.

From the fact that phosphates played a very important part in the regulation of neutrality of the body, the question arose whether phosphates did not also assume an important rôle in the elimination of acids from the body. Fitz, Alsburg and Henderson gave large doses of acid to rabbits for several days at a time. They observed marked increase in phosphate excretion and drew the conclusion that phosphates did play a very important part in the elimination of acid from the body. Their work has already been adversely criticised above on the grounds that they used doses far too large for the small animals used and gave the acid for very prolonged periods, the secondary effects resulting, vitiated their experiments.

An increase in phosphate excretion by the kidney had been observed, on numerous other occasions by various authors, after the administration of acid to various species, especially to the human subject. All this has contributed to the belief that increased phosphate excretion played a very important part in the elimination of acid. But the workers - Steenbock, Nelson and Hart, Givens and Mendal and Zucker - who have determined phosphorus content of faeces and urine, before and after acid administration,

have found the increase in phosphate excretion by the kidney always accompanied a corresponding diminution in the excretion of phosphate by way of the intestines. The work quoted from literature is enough to show that the belief so widely held is not based on proved facts.

Though technical difficulties render it impossible to prove beyond doubt that the faeces lose exactly to the same extent as the urine gains, it must be held as proven that diversion of phosphates from the intestine to the urine does take place after acid administration. There is in the absence of actual analytical data, including both urine and faeces, hardly any reason to doubt that the increase in phosphate in urine after acid administration is due solely to this fact. It seems to us, therefore, particularly significant that our fasting animals, including those which received acid by paths other than the digestive tract, show no change in phosphate excretion. The same is confirmed by the ammonia and residual fixed base figures.

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(5) S U M M A R Y.

Being convinced that dietary influences have been a prolific source of confusion in the past, we decided to use fasting subjects. We had also come to the conclusion that the rôle played by fixed base in the neutralisation of acid can be more accurately studied, if some acid other than hydrochloric acid is used. We chose sulphuric acid. In choosing maximum dose we were guided by the normal daily acid production in fasting cats - our experimental subjects. This also corresponds to the magnitude of the excess acid production in phorhizinized dogs and cats. We did not wish to get too far away from the rate at which acid may be formed in the tissues spontaneously, so that our results may be expected to have more bearing on the fate of acid produced under natural conditions than is the case with some previous work.

Acid per Os.

Sulphuric acid was given from 30 c.c. to 12·5 c.c. per kilogram weight in single doses to starving cats. About 80% of the total amount of the acid given was recovered from urine as sulphate. It was found that ammonia neutralised about 60% of the acid given by the mouth and subsequently recovered from the urine, or 50% of the total amount given. This figure for ammonia is very

much higher than (20 or 30%) had been observed in experiments performed on animals that had been fed during the experiments.

Varying proportions of the acid were neutralised by fixed base. Taking the extreme case noted by us, the amount of fixed base lost is 55 c.c. of 0·1N base in a cat of 2·55 kg. weight. This corresponds to a loss equivalent to 6·7 volume per cent carbon-dioxide, - a small fraction of the normal range of variation in the bicarbonate content of plasma. If acid feeding deprives the body of fixed base at all, it draws only on a supply which is not particularly needed and which is, therefore, not tenaciously retained.

No appreciable change in phosphate excretion was noted in any of the experiments.

Parenteral Administration of Acid.

Intravenous injection.

Approximately the same dose of sulphuric acid was given as per Os. To approximate the rate of absorption when acid is given by mouth, the acid was given at from about 0·015 c.c. per kilogram per minute to about 0·09 c.c. per kilogram per minute. It was noted that little or no increase in ammonia took place, and that a sufficient rise in residual fixed base excretion occurred to account for all the acid injected. But in every experiment profound secondary changes occurred, chief among which were

haemolysis interfering with the action of the kidney and hyperpnoea. The latter alone would explain the apparent absence of ammonia production; over-ventilation evidently setting free more than enough fixed base to neutralise the acid, so that ammonia production was rendered unnecessary. We were forced to the conclusion that this method of parenteral introduction of acid defeated the object we had in view, i.e. to see whether acid entering the systemic circulation directly is neutralised by ammonia at all. Such neutralisation had been denied by Keeton. We therefore tried parenteral administration under less severe conditions, i.e. Subcutaneously, and it served the purpose well.

100 per cent. of the acid when administered by this route was recovered from the urine. Increased ammonia production neutralised about 40% of the acid. This average is based on two experiments only, while the average for alimentary route administration is based on a very much larger number of experiments, and we are not sure the causes of the peculiar results of intravenous injections are wholly absent here.

No increase in phosphate excretion in urine was noticed in response also to the parenteral administration of acid.

C O N C L U S I O N.

1. The principal means that the organism possesses for neutralising acid is Ammonia production.

2. The mechanism of Ammonia production is independent of the mode of entry of acid into the circulation, i.e. whether via the liver or directly into the blood-stream, and is consistently brought into play, even after the exhibition of comparatively small single doses of acid.

3. If acid administration in small doses, comparable to those produced spontaneously by the tissues, deprives the body of fixed base at all, it draws only on a supply which the tissues do not need, and can, therefore, easily spare. This loss corresponds to a small fraction of the normal variation in the bicarbonate content of the plasma.

4. Administration of acid per os, or parenterally, does not cause any increase in the phosphate excretion in the urine.

The author wishes to acknowledge his indebtedness to Dr. C. H. Fiske for his advice and interest in this investigation.

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