XCIV. THE EFFECT OF THE CONTINUED INGESTION OF MINERAL ACID ON GROWTH OF BODY AND BONE AND ON THE COMPOSITION OF BONE AND OF THE SOFT TISSUES.

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STRONG acid introduced into the animal body appears to be mainly excreted in combination with ammonia, but evidence exists to show that some disturbance of the relative proportion of the fixed bases in the blood is produced [Stewart and Haldane, 1924; Allers and Bondi, 1907], and that some extra excretion of the fixed base of the body may be brought about [Steenbock, Nelson and Hart, 1914; Givens and Mendel, 1917; Stehle, 1917; Lamb and Evvard, 1919; Shohl and Sato, 1923; Hunter and Aub, 1927]. The nature of the extra fixed base thus excreted would seem to be determined by the excess of available base, which depends on the diet [Lamb and Evvard, 1919; Stehle and McCarty, 1921].

Gamble and Ross [1923] concluded that most of the extra base excreted after the administration of acid was merely washed out in combination with the usual acids of the body as a result of the diuresis which follows such administration, but Fiske, Goodell, Hathaway and West [1926] found that fixed base was excreted in excess of the base merely washed out in this way. After this preliminary loss of base, however, they noted a subsequent retention. All these conclusions were drawn from short metabolism experiments. Goto [1918] examined rabbits to which acid had been administered for various periods. He found a great loss of weight, a loss of fat and carbonate, but not of calcium of bone and a reduction in the average potassium and phosphate content of the muscles of the acid group as compared with the controls. The latter varied greatly among themselves, however, and the two groups overlapped considerably. The rabbits were adults of varying age and breed.

Lamb and Evvard [1919] examined the bones of a pig to which they had administered acid for a long period. The amount of marrow was much reduced. No chemical examination was made.

EXPERIMENTAL.

The following experiment was carried out to determine whether acid ingestion exerted any influence on the storage of base in the tissues of young growing rabbits and rats. The animals used for the experiment were all bred in the laboratory from stock kept on standard diets, and were reared under carefully controlled conditions; animals which received acid were only compared with litter-mate controls. It was hoped in this way to detect small changes in bone and tissue composition.

Their age at the beginning of the experiments was about 6 weeks, but such great difficulty was experienced in rearing more than one or two rabbits in each litter, that the later experiments were carried out on rats.

Diet. The diet of the rabbits consisted of oats, and bran mash with lettuce or cabbage *ad libitum*.

The rats received Steenbock's stock diet [Bethke, Steenbock and Nelson, 1923], with fresh milk and water *ad libitum*.

Administration of acid. The acid used was 0.1 *N* hydrochloric acid. In the first experiment, carried out on two rabbits, with one litter-mate control, the acid was given by stomach tube, but this caused acute local lesions in the stomach and one rabbit had to be discarded. At the end of 17 days the second animal died. The bones of this animal and of its control were taken for chemical examination.

In all later experiments, the acid was administered by mixing it with the mash in the case of the rabbits, and with the maize mixture in the case of the rats. Under these circumstances it was not possible to know accurately the amount of acid consumed, as some scattering of food always took place, but an approximate estimate of the average acid consumption per animal per day could be arrived at.

Examination of the tissues.

(a) *Bones.* In the rabbits, a femur, fibula and tibia, humerus, radius and ulna were dissected out, freed as far as possible from connective tissue and analysed separately for ash, fat, water, organic residue, calcium and phosphorus. No specific difference was, however, found in the composition of the different bones, and the results for the femur, fibula and tibia are combined and presented together as typical of the bone composition in each animal.

In the rats all the leg bones of the four legs in each animal were used for the analyses.

(b) *Soft tissues.* The muscles of the hind legs of the rabbits were removed and freed as far as possible from fascia (no attempt was made to free the muscles from blood vessels, nerves, etc.). The water content was determined by drying at 110° for 48 hours. The muscles were then ashed and the total potassium and sodium were estimated by the method of Husband and Godden [1927]. By this method the total potassium and sodium were weighed as the

mixed sulphates. In the mixture the sulphate was determined and from this the base equivalent to potassium + sodium was calculated as cc. of normal base per 100 g. of muscle tissue. The heart, lungs, kidneys and liver were weighed together, and the water and total potassium + sodium were determined on these tissues as for the muscles.

In the case of the rats it was not practicable to make these determinations on the muscles alone, and the analyses were made on the leg muscles and organs taken together.

Experiments on rabbits.

Three experiments were carried out on rabbits from four litters. Only one rabbit was available from litter 3 which was about the same age as litter 2, but of a larger breed. The results are shown in Table I.

The animals receiving acid either lost weight whilst their controls remained steady, or gained weight much less rapidly than their controls. There were two exceptions. In litter 2, the control animal lost 50 g. while the experimental animal lost 150 g. and in litter 4 one animal receiving acid grew as well as its controls. The control of litter 2 was not suffering from any specific disease and its failure to grow was unaccounted for. The ratio of final bone weight to body-weight indicated that the growth of bone had been rather less retarded than body growth, *e.g.* in litter 4 the final bone weight expressed as a percentage of final body-weight was 0.64 for the two controls, and 0.65 for the experimental animal which grew well, and 0.75 and 0.71 for the experimental animals which showed diminished growth.

The only significant changes in the chemical composition of the bones of the acid group were the increase in the percentage of water and decrease in the percentage of organic matter. Where analyses of fat were made it was found that the decrease in organic matter occurred in the fatty moiety. The control of litter 2 had a bone composition which, like its body growth, was typical rather of the acid than the control group, while the member of litter 4, which grew as well as its controls, had bones of composition similar to those of the controls.

It would therefore seem that the most striking effect of acid ingestion in rabbits is a loss of weight or diminution in growth rate, and that the influence on bone composition is merely that of marked malnutrition (*i.e.* loss of fat and increase in water of bone [Korenchevsky, 1922; Jackson, 1925]). The two animals in the fourth litter, in which growth was retarded, showed a very slight reduction in ash and calcium content of the bone as compared with their controls, but the significance of this is doubtful.

Analyses of the tissues. Table I gives the water content, and the base content of the fresh and dry muscles and organs. From these figures it would appear that there was a slight but definite reduction in base content (sodium + potassium) in fresh muscle in rabbits after the ingestion of acid. This difference was not regularly found in the dry tissues, owing to variations in the water content.

Table I.

Litter	Duration of exp. days	Amount of N HCl administered cc.	Bone analysis										Soft tissues					
			Bone wt. (g.)	Ash % (A)	Water %	Fat %	Organic residue % (R)	Ca %	P %	Ca/P	100 g. muscle		100 g. organs		Water %			
											Initial	Final	Fresh	Dry	Fresh	Dry	Muscle	Organs
1	17	0	6.714	26.7	39.2	13.8	20.2	9.6	—	—	13.0	51.4	12.5	46.0	74.8	72.8		
2	17	45	7.151	25.7	50.1	5.0	19.2	9.3	—	—	11.0	43.8	10.4	43.4	74.8	76.1		
	28	0	4.400	21.0	58.1	1.4	19.4	7.7	4.2	1.85	12.9	61.1	12.4	51.2	78.9	75.8		
3	28	120-150	5.108	20.6	59.9	0.7	18.8	7.5	4.1	1.85	11.3	48.8	13.5	60.3	77.0	77.6		
	28	120-150	6.668	28.6	48.4	—	23.0*	10.5	5.2	2.00	14.2	62.0	11.1	42.9	77.1	74.1		
4	32-35	0	4.131	19.0	47.8	—	33.2*	6.5	3.6	1.82	14.7	54.4	14.0	50.0	73.0	72.0		
	32-35	0	3.515	19.0	52.3	—	28.7*	6.5	3.6	1.82	14.5	64.2	12.5	53.2	77.4	76.5		
	32-35	150-200	2.994	16.8	67.9	—	15.3*	5.9	3.3	1.80	12.9	64.4	13.1	59.5	80.0	78.0		
	32-35	150-200	3.546	15.7	66.8	—	17.5*	5.4	3.0	1.82	11.7	54.4	11.0	54.4	78.5	79.8		
	32-35	150-200	3.881	22.0	50.4	—	27.6*	7.7	4.0	1.92	8.6	39.6	9.8	35.5	78.3	72.4		

* Organic residue + fat.

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Table II.

Litter	Duration of exp. days	Amount of N HCl administered cc.	Final body-weight (g.)	Bone analysis							Soft tissues			
				Bone weight (g.)	Ash % (A)	Water %	Fat %	Organic residue % (R)	Ca %	P %	Base equiv. (cc. N alkali) per 100 g. fresh tissues			
											Ca/P	A/R	Water %	
A	70	0	118	2.461	38.9	31.3	6.7	23.1	14.5	7.2	1.69	2.03	12.5	73.9
	70	0	114	2.510	34.5	37.5	5.6	22.4	13.0	6.3	1.54	2.05	12.1	75.3
	70	0	110	2.269	39.8	31.8	5.2	23.2	15.4	7.3	2.10	1.72	13.0	72.6
	70	0	104	2.313	37.8	33.4	6.5	21.8	14.3	7.0	2.03	1.73	12.3	73.2
	Mean	...	112	2.388	37.8	33.5	6.0	22.6	14.3	6.95	1.67	2.05	12.5	73.7
A	70	100-200	...											
	Mean (4 rats)	...	74	1.971	36.7	34.8	4.0	24.5	14.0	6.90	2.02	1.50	13.8	74.0
B	60	0	...											
	Mean (4 rats)	...	105	1.944	37.6	34.4	4.9	23.0	14.7	7.0	1.64	2.10	12.4	73.6
B	60	100-200	...											
	Mean (4 rats)	...	83	1.745	36.9	34.8	4.1	24.2	14.4	7.0	1.52	2.05	12.9	74.1

The variations in the figures for the rats in the control group, litter A, indicate the order of the variations found in the different groups.

There was not, however, evidence of regular reduction in water content of muscle after prolonged ingestion of acid, as might be expected from the diuresis following the ingestion of small quantities of acid.

In the organs there was no regular decrease in base content either in fresh or dry tissues. Again, the water content of the organs from the acid group was usually though not invariably higher than in the controls. (The liver and kidneys of the experimental animals always appeared gorged with blood after the death of the animals.) The analyses of the tissues of the one animal which grew as well as the controls (litter 4) indicated a much more considerable withdrawal of base from the soft tissues than in any of the others, although the bones of this animal were less affected by the acid ingestion.

From these analyses it would therefore appear that acid ingestion in rabbits, as found by Goto [1918], was followed by a withdrawal of base from the muscles. There was no evidence that a withdrawal of base from the organs usually occurred, although from the one rabbit which grew well it may be deduced that such a withdrawal may occur in certain animals under certain conditions. The feeding of the rabbits may have somewhat complicated their response to the acid feeding. As cabbage was supplied *ad libitum* it is possible that some animals may have chosen to eat more than others. This may have accounted for the variations found in the acid group of the fourth litter.

Experiments on rats.

Two litters, each containing eight rats, were used. Four rats from each litter were given acid and four were kept as controls. Both litters contained even numbers of the sexes. The rats each received about 12 cc. of acid daily (0.1 *N* HCl) at the beginning of the experiment. This was gradually increased until at the end of 7 weeks they were each receiving 50 cc. of 0.1 *N* acid daily, and then for the last 2 weeks they were given 50 cc. of 0.2 *N* acid each per day. After the dose of acid reached 25 cc. 0.1 *N* per day, the appetites of the animals fell off and the food supply to the controls was gradually limited to approximate to the amount actually consumed by the experimental groups. This brought the rate of growth of the control animals well below the average for their age, but made it possible to determine the influence of the simple ingestion of acid by the experimental rats, uncomplicated by differences in food intake. After 9 weeks on experiment the animals died, the controls were killed and the bones and tissues were examined as previously described.

The effect of acid ingestion on bone and body growth in rats. Table II shows the final body-weight, bone weight and bone composition of the rats receiving acid and of their controls. The bones (of the four legs) were on the whole slightly smaller in the groups receiving acid than in the control groups. The final body-weights were markedly smaller in the acid than in the control groups with the exception of one rat in litter B. Some of the rats in the experimental group grew as fast as the controls at first, and then lost weight rapidly in the final 2 weeks of the experiment; others grew more slowly than

the controls throughout the whole period. The average bone weights of the groups expressed as percentages of the maximum body-weights were: litter A, acid group 2.08, controls 2.13; litter B, acid group 1.96, controls 1.85. This would seem to indicate that bone growth had been approximately proportional to body growth in both groups and that the final loss of weight in some of the experimental animals had not been accompanied by appreciable breakdown of bone. On the whole, however, both bone and body growth had been slightly retarded in the acid groups.

Bone composition. From Table II it may also be noted that the ingestion of considerable quantities of acid was without appreciable effect on the chemical composition of the bones. The percentages of calcium and phosphorus in the ash of the bone did not indicate any differences between the bones of the two groups. The A/R ratio which may be taken as a measure of the degree of ossification of the bone [Chick and Roscoe, 1926] was slightly but definitely decreased in the acid group, although the animal in litter B, which grew as well as its controls, also had an A/R ratio which fell into the range of the control group. Each of the control groups also contained one animal of which the A/R ratio was typical rather of the acid than control groups. Despite this variation the difference between the two groups, although slight, would seem to indicate a definite retardation in the rate of ossification of bone in the acid group, particularly during the last few weeks of the experiment. The marked reduction in fat content of the bone which was so typical of the rabbits receiving acid was not found in the case of the rats on the acid diet.

Analysis of the soft tissues. The soft tissues of the animals in the acid group showed no loss of water as compared with their controls. There was no diminution in the percentage of base in the wet tissues of the acid group (Table II). On the contrary, there was a slight increase in this respect in all the animals receiving acid, with the one exception of the animal in litter B which grew normally. Not only in growth but in composition of bone and tissues this animal appeared to be similar to the control rather than the acid groups.

DISCUSSION.

While in the muscles of rabbits receiving acid there seemed to be a slight reduction in base content (potassium + sodium), this was not the case for the organs of the rabbit, nor would it appear to be the case for the muscles and organs of the rat. The most striking influence of the ingestion of acid was the chronic malnutrition of the animals resulting in retarded growth and in six out of seven rats considerable loss of weight. The effect on the bones of rabbits was also that typical of severe malnutrition. In the case of the rats, it may be concluded from the A/R ratio of the different groups that the ossification of bone was retarded when the ingestion of acid was so great that actual loss of weight occurred, but there was no sign in any animal of a differential withdrawal of calcium from bone, resulting in softened bones, nor of a breakdown

of osseous tissue resulting in bones which were of small weight compared with the weight of the animal. The tissues of the animals in the acid group showed no signs of dehydration, as might have been expected from the diuresis shown in the short period experiments of Fiske and his co-workers [1926] and Gamble and Ross [1923].

If it were assumed that the excess acid was carried in the blood as ammonium chloride, it might be concluded that the retardation of growth and final loss of weight was due to the toxicity of this ammonium chloride. During the last weeks of the experiment the animals daily ingested enough acid to produce a lethal dose of ammonium chloride [Underhill and Kapsinow, 1922], but the effect of the latter would be minimised by being slowly produced and by being excreted as formed. Gamble and Ross [1923], however, showed that the ingestion of ammonium chloride had the same influence on the composition of blood and urine (p_H , serum-bicarbonate, ammonia of urine, etc.) as the ingestion of an equivalent quantity of hydrochloric acid. They concluded that the ammonium chloride was dissociated and that the ammonia was carried in the blood as some neutral substance, while the hydrochloric acid was carried in combination with the fixed base of the serum, and the serum-bicarbonate was lowered. In the case of prolonged ingestion of acid, however, this mechanism might prove inadequate, as plasma-bicarbonate could not be lowered beyond a certain point if tissue CO_2 was to be removed satisfactorily. An increase in plasma-base would therefore be necessary to permit of the transport of the excess acid. (This increase in total plasma-base might be achieved by increasing the plasma volume without altering the concentration of base in the plasma.) The net result would be a greater call on the bases absorbed from the food to supply plasma-base, and a limitation of base available for tissue building.

From the colloidal nature of cell protoplasm, it would seem probable that the salts present in the tissues play an important part in preserving the electrical equilibria which are the conditions necessary for the normal functioning of the cells, and that, without an adequate supply of salts available for tissue-building, normal growth cannot take place. Hence the limitation of growth where the conditions tend to increased base excretion. In the rats, when the dose of acid was greatly increased towards the end of the experiment, actual breakdown of tissue occurred, presumably to supply the base necessary for the neutralisation and excretion of acid. This liberated, however, more base than was actually necessary for reaction with the acid, and some was retained in the tissues. This would account for the slightly increased base content in the soft tissues of those rats of the acid group in which considerable loss of weight had occurred. This retention of base in the tissues during the rapid breakdown of other tissues may perhaps be comparable to the retention of sodium in starvation [Lusk, 1928].

SUMMARY.

(1) Five rabbits on a fixed diet received acid daily for periods varying from 17 to 35 days. Compared with litter-mates on the same diet without acid, these animals showed a reduction in the base content of the muscles, but not of the viscera.

(2) There was no reduction in the percentage ash of the bones, but a marked reduction in the fat content.

(3) All the animals receiving acid showed either retarded growth or actual loss of weight, and suffered from severe malnutrition.

(4) Eight rats on a standard diet received daily doses of acid over a period of 9 weeks.

(5) There was no appreciable reduction in the fat content of the bones, and the ash content was unchanged.

(6) Growth was retarded, and considerable loss of weight occurred when the dose of acid was increased.

(7) The base content of the soft tissues was increased slightly where death was preceded by marked loss in weight.

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