Onderstepoort J. vet. Res. 37 (3), 151–156 (1970)

CHANGES IN THE LEVELS OF PLASMA ELECTROLYTES AND GLUCOSE IN SEVERE ARTIFICIALLY INDUCED ACIDOSIS IN MERINO SHEEP

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ABSTRACT

PROCOS, J., SCHUBERT, ANNETTE & BRIEL, B. J. Changes in the levels of plasma electrolytes and glucose in severe artificially induced acidosis in Merino sheep. *Onderstepoort J. vet. Res.* 37 (3), 151–156 (1970).

Severe acidosis was induced in wethers by the infusion of mineral and organic acids. No clinical signs apart from slight panting, were observed despite a fall in plasma glucose levels which occurred either spontaneously during the infusion of acetoacetic acid, or artificially following administration of insulin in conjunction with hydrochloric acid infusions. It is suggested that acidosis *per se* is not immediately responsible for the induction of the nervous symptoms displayed by sheep suffering from pregnancy disease.

INTRODUCTION

The problem of acidosis in sheep and its relationship to the nervous symptoms of pregnancy disease have received very little attention, even though there have been references to sheep developing an acidosis while suffering from pregnancy disease (Sampson, Gonzaga & Hayden, 1933; Cameron & Goss, 1940). Considerable controversy exists as to whether the nervous symptoms in human diabetic ketosis are the result of the toxic effect of the ketone bodies, or are due to the acidosis which invariably results from the presence in the blood of high concentrations of these metabolites. In sheep, on the other hand, the nervous symptoms of pregnancy disease have been mainly ascribed to the hypoglycaemia which is a prominent feature of this disorder (McClymont & Setchell, 1955). Nevertheless, the possible deleterious effect of the accompanying acidosis on the central nervous system has by no means been excluded, particularly as the appearance of clinical signs amongst pregnant ewes has been found to be invariably preceded by both persistent ketosis and hypoglycaemia (Procos & Gilchrist, 1966).

The present paper describes the clinical signs as well as the biochemical findings obtained in artificial acidosis induced in normal sheep by the intravenous infusion of acids for short or longer periods of time. Infusions of hydrochloric (HCl) as well as acetoacetic (AA) and betahydroxybutyric (BHB) acids were carried out in order to divorce the effect of the acidosis produced by the mineral acid from the combined effect of acidosis and ketosis induced by the two organic acids. This was especially important since Bergman, Kon & Katz (1963) had previously demonstrated that sodium acetoacetate administered intravenously is capable of inducing a hypoglycaemia as well as nervous signs in non-pregnant sheep.

No studies on acid-base metabolism have been reported for Merino sheep, and normal values of plasma electrolytes in the literature have not been done simultaneously on the same sheep. In view of the very close interdependence between these electrolytes, the electrolyte status of an animal can be most clearly defined when they are determined simultaneously. Thus prior to the present investigation, normal values of the five main plasma electrolytes (Na^+, K^+, Cl^-, HCO_3^-, Protein) were obtained simultaneously for 49 normal Merino sheep including those used in the infusion experiments.

MATERIALS AND METHODS

Animals

The 49 well-nourished, adult Merino wethers and ewes listed in Table 1 were employed for the determination of the normal values. Twenty-four of these wethers were also utilized in the infusion experiments. Four (K7, P2, P25, D40) died in preliminary experiments and the rest are listed in Table 3. The sheep were fed a diet of lucerne hay and mineral/trace-element lick *ad libitum*, for at least a year before the start of the experiment. They weighed approximately 55 kg.

Acids infused

HCl: The 0.05, 0.10, 0.25, 0.50, 1.80 and 3.60 N solutions were made up in physiological saline (0.9 per cent NaCl).

AA: An 0.25 N (2.60 per cent) solution was prepared by alkaline hydrolysis of ethyl-AA (Bergman *et al.*, 1963) followed by acidification with 2 N HCl. The resulting concentration of NaCl in the solution was 1.46 per cent. An 0.83 N (8.63 per cent) solution was similarly prepared but the cation was removed by trituration with the appropriate amount of Dowex G50 W-XB (50 to 100 mesh) cation exchange resin. The solution was made up to volume with physiological saline.

The AA solutions remained stable at room temperature for at least 12 hours.

BHB: An 0.25 N (2.60 per cent) solution was prepared by acidifying a solution of Na-BHB with 2 N HCl. The concentration of NaCl in the solution was 1.46 per cent.

Methods of administration

(*A*) *Short-term infusions:* The acids were infused into the jugular vein of the experimental animal by means of a temporary polythene catheter and a 100 ml burette. The rate of dosing was, in every instance, 20 ml/min, while the total infusion time was 10 min.

(*B*) *Long-term infusions:* Infusions were performed by means of a Bühler infusion pump (Type mp[1]) and a temporary catheter in the jugular vein. In this case infusion rates of 2 to 8 ml/min were employed, and the infusion time was 6 to 8 h.

*Employed by the Stock Diseases Fund of the Meat, Dairy and Wool Boards of the Republic of South Africa

Received 12 June 1970

Sampling

(*A*) *Normal values:* Jugular blood samples (12 ml) were all obtained on the same day at 8 a.m. before feeding. The blood was collected into 15 ml centrifuge tubes containing heparin which were hermetically closed to just above the blood level with a rubber stopper. The tubes were immediately centrifuged for 20 min at 2800 r/m and the resulting plasma was used for the chemical determinations.

(*B*) *Short-term infusions:* Samples of blood (12 ml) were obtained from the jugular vein on the opposite side from the one through which the infusion was given. The samples were taken immediately before, during (at 6 min after the infusion was started) and after the infusion (at 2 and again at 30 to 40 min after the infusion was completed).

(*C*) *Long-term infusions:* Jugular blood samples were collected immediately before the infusion and thereafter at intervals of 1 to 2 hours as described above.

Analytical methods

Plasma chloride was measured by the titrimetric method of Schales & Schales (1941). Total plasma protein was estimated by the biuret method of Weichselbaum (1946). An EEL flame photometer was used to determine sodium and potassium concentrations, while the titrimetric method of Van Slyke & Neill (1924) was utilized to measure plasma bicarbonate levels. All the values were converted to milliequivalents per litre (meq/l).

Plasma glucose was determined by the oxidase-peroxidase method (Böehringer, Mannheim GmbH, W. Germany) and expressed as milligrams per cent.

Results

Normal values

Details of the normal values obtained for each sheep are presented in Table 1. The means and ranges of the values found by us, together with similar values obtained for humans and dogs by Carr & Schloerb (1959), are presented in Table 2. Comparison of the figures indicates that, apart from protein, our electrolyte values for sheep are in much closer agreement with those observed in humans than those obtained for dogs. Our range of values for sodium and potassium respectively (132.5 to 166.5; 4.26 to 5.98) is in agreement with the range of normal values for sheep obtained by Clark (1959) (132 to 156; 3.8 to 6.0) and Pierce (1959) (140.5 to 157.5; 4.5 to 5.9) but somewhat lower than that found by McClymont, Wynne, Briggs & Franklin (1957) (149.2 to 154.4; 5.69 to 6.24). No reference in the literature could be found as to the range of normal values for bicarbonate in sheep.

A. short-term infusions

(1) *Plasma electrolytes:* As very little was known about the reaction of sheep to intravenous infusions of

Table 1 Normal values (meq/*l*) of plasma electrolytes

Sheep No.	Sex	Protein	Cl^-	HCO_3^-	Na^+	K^+
P36	E	24.59	—	28.60	144.03	5.18
P34	E	22.99	100.86	28.45	160.40	4.99
P41	E	22.99	110.43	26.70	145.96	4.45
P31	E	24.59	95.78	31.70	148.28	4.26
P23	E	22.82	103.97	27.25	144.03	5.18
P17	E	25.39	100.52	27.55	148.28	4.88
P33	E	20.80	103.39	26.97	141.90	5.12
P46	W	22.72	100.95	27.60	152.53	4.80
P80	W	20.85	108.50	28.15	148.28	4.80
P45	W	23.16	100.00	26.30	144.03	5.55
P53	W	22.72	105.67	26.22	152.53	5.80
P44	W	21.65	99.48	31.05	158.90	4.51
P57	W	23.52	105.85	25.12	144.12	5.18
P48	W	20.31	106.12	23.50	—	5.12
P56	W	25.13	113.54	27.50	139.78	5.12
P19	W	25.13	110.18	26.80	148.28	5.00
P25	W	22.45	107.06	26.70	137.65	5.00
P 6	W	21.34	111.02	26.70	141.90	4.51
P21	W	22.45	—	—	152.53	5.37
P55	W	22.45	113.46	27.20	—	5.12
P49	W	22.40	101.53	29.45	—	5.25
P 9	W	21.92	112.53	24.70	148.28	4.80
P43	W	19.35	106.86	24.27	145.96	5.86
P47	W	23.48	111.02	26.45	141.90	4.82
P12	W	20.80	108.49	28.85	152.53	5.00
P15	W	21.38	105.93	27.27	148.28	5.83
P10	W	20.22	99.22	27.00	145.96	4.94
P65	W	24.38	—	25.67	144.03	5.49
P27	W	21.82	105.34	23.62	139.78	5.12
P16	W	20.22	106.14	29.30	150.40	4.94
B1	W	19.78	107.46	27.02	145.91	5.18
B2	W	21.82	105.08	25.52	135.20	5.00
B3	W	20.75	106.95	26.52	142.34	5.83
B4	W	20.84	103.47	27.80	153.06	5.68
K 3	W	22.45	113.79	25.71	160.20	5.83
K 6	W	20.58	113.36	25.51	156.63	4.82
K 9	W	19.03	111.81	23.31	—	—
K16	W	19.03	108.71	27.76	149.48	4.75
K17	W	23.69	111.21	26.61	153.06	5.12
K20	W	20.41	111.38	27.57	156.63	5.80
K45	W	20.85	112.33	28.11	156.63	5.25
K54	W	21.91	105.34	27.67	142.34	—
K56	W	22.99	110.09	29.76	163.77	5.37
K60	W	22.02	109.48	28.51	153.06	5.83
K67	W	21.29	111.29	26.36	145.91	5.98
K30	W	20.75	103.92	26.40	142.52	4.90
*K30		17.74	105.28	21.99	138.52	—
*K30		19.13	102.48	23.62	138.52	4.40
*K30		20.26	108.79	24.40	138.52	5.00
*K30		19.40	103.30	26.73	138.52	5.55
K41	W	19.90	104.90	27.73	146.52	5.60
*K41		19.73	103.30	28.25	138.52	4.40
*K41		20.86	100.72	25.42	166.52	5.45
*K41		20.76	99.68	27.88	—	—
K51	W	26.17	99.92	27.52	164.52	4.90
*K51		25.27	99.12	27.67	—	—
*K51		24.91	104.26	28.23	151.52	5.50
*K51		22.97	105.08	27.35	137.52	4.95
*K51		23.65	102.50	26.53	132.52	4.40
K39		19.81	107.52	27.44	137.52	4.20
*K39		20.35	102.37	23.45	—	—
*K39		20.85	100.72	27.78	137.52	4.80
*K39		20.91	106.25	27.55	132.52	4.80

*Values obtained on successive days
E = Ewes
W = Wethers

Table 2 Values of plasma electrolytes of normal dogs, humans and sheep

Plasma electrolytes	Dogs*		Humans*		Sheep	
meq/*l*	Mean	Range	Mean	Range	Mean	Range
Na^+	148.8	130—163	143.0	128—148	146.7	132.5—166.5
K^+	3.8	2.3—5.1	4.4	3.6—5.3	5.11	4.26—5.98
Cl^-	111.0	87—120	106.0	95—108	105.98	95.78—113.79
HCO_3^-	20.6	15.7—25.9	26.1	22.6—30.8	26.88	21.99—31.70
Protein	15.8	12.2—20.9	17.2	14.1—20.5	21.81	19.03—26.17

*Carr & Schloerb (1959)

TABLE 3 Means and ranges of plasma electrolyte values (meq/l) obtained for short-term (10 min) infusions (200 ml) of 0.50 or 0.25 N HCl and of 0.25 N AA or BHB acids

Acid infused	Sheep No.	No. of exp.	Plasma electro-lytes	Pre-infusion sample		6 min after infusion started		2 min after infusion completed		30-40 min after infusion completed	
				Mean	Range	Mean	Range	Mean	Range	Mean	Range
0.5 N HCl in an 0.9% NaCl solution	K30, K41, K51	4	Cl^- HCO_3^- Protein	101.00 26.26 22.48	99.12–105.28 21.99– 27.88 17.74– 25.17	107.45 18.95 22.10	101.36–113.51 15.24– 20.77 17.74– 25.27	110.04 13.51 22.99	103.92–118.31 7.94– 17.44 17.51– 27.53	107.27 18.98 21.33	102.16–113.51 18.84– 22.77 44.31– 25.36
0.25 N HCl in an 0.9% NaCl solution	K8, K30, K39 K41, K48, K51	15	Cl^- HCO_3^- Protein Na^+ K^+	106.38 26.38 20.60 141.80 4.93	100.72–110.14 23.62– 28.85 19.13– 24.91 132.52–159.40 4.20– 5.60	109.10 22.16 21.24 138.23 4.98	104.96–113.81 17.15– 23.70 17.55– 25.30 129.52–142.52 4.30– 6.60	112.23 18.70 21.37 138.42 4.93	105.52–116.26 15.09– 24.00 18.68– 28.69 132.52–146.52 4.20– 5.55	110.19 21.96 20.08 144.52 4.60	104.72–117.17 20.20– 24.85 18.59– 22.97 136.52–151.52 4.40– 5.40
0.25 N AA acid in a 1.46% NaCl solution	K8, K48, P9 P17, P23, P27 P33, P41, P44 P46, P47, P57	12	Cl^- HCO_3^- Protein Na^+ K^+	107.47 24.75 20.96 159.58 4.63	103.19–110.48 19.20– 28.18 18.66– 25.13 153.70–164.81 3.89– 6.52	109.84 21.36 19.89 159.78 4.30	107.33–113.71 16.19– 26.43 18.01– 24.32 153.70–170.52 3.52– 6.41	112.04 20.01 19.53 160.94 3.76	108.95–117.72 15.80– 24.81 16.48– 25.66 157.40–164.81 3.38– 4.55	113.58 22.41 20.19 164.53 4.32	108.61–118.23 12.63– 26.30 18.27– 21.48 159.26–170.37 3.66– 5.45
0.25 N BHB acid in a 1.46% NaCl solution	K1, K8, K19 K30, K41, K48 K51, P23, P57	10	Cl^- HCO_3^- Protein Na^+ K^+	107.73 24.85 21.53 153.30 4.46	102.61–113.25 22.63– 27.74 19.25– 25.39 149.63–155.56 3.80– 5.16	109.04 21.34 20.25 152.26 3.90	103.04–115.26 18.10– 25.43 17.80– 22.99 150.00–157.40 3.43– 4.18	109.67 20.43 19.46 152.77 3.89	105.31–113.51 17.51– 22.90 18.18– 22.45 150.00–159.26 3.28– 4.56	110.65 22.55 20.06 153.24 4.18	106.90–117.58 17.75– 26.19 17.42– 21.92 150.00–161.11 3.54– 5.12

hydrochloric acid, the concentrations which could be used to induce severe acidosis without causing the blood to coagulate or to haemolyze were ascertained in a series of preliminary experiments. For short-term (10 min) infusions where the infusion rate was 20 ml/min, the range of concentrations used was 0.25 to 0.50 N.

The average values and ranges of plasma electrolytes obtained for infusions of 200 ml of 0.50 and 0.25 N HCl in 0.9 per cent saline as well as 0.25 N BHB and AA acids in 1.46 per cent saline are presented in Table 3. In every case the concentrations of Cl^-, HCO_3^-, protein, Na^+ and K^+ in pre-infusion plasma samples fell within their normal ranges reported in Table 2. Thus in this respect every animal was normal prior to infusion. Following the infusion of the three acids no significant changes in plasma electrolytes were observed apart from a drop in bicarbonate levels. As expected, infusion of 0.50 N HCl induced a far greater degree of acidosis than either the 0.25 N HCl or the 0.25 N BHB and AA acids. In the case of 0.50 N HCl, the average minimum plasma bicarbonate level was 13.51 (range 7.94 to 17.44) meq/l as against 18.38 (15.09 to 21.20), 20.43 (17.51 to 22.90) and 20.01 (15.80 to 24.81) meq/l for 0.25 N HCl, BHB and AA acids respectively. Although a pH value of 3.5 was obtained for each of the 0.25 N solutions of the three acids, thus indicating that they were indeed equivalent in respect of hydrogen concentration (50 meq/l), the depth of acidosis induced by the HCl was somewhat greater than that brought on by the BHB or AA as indicated by the plasma bicarbonate values. This discrepancy was most probably due to the differences in rates of utilization and excretion between the mineral acid on the one hand and the two organic acids on the other.

The concentration of sodium chloride (1.46 per cent) in the 0.25 N BHB and AA solutions was calculated to be higher than that of physiological saline (0.9 per cent). This might have influenced the concentration of the plasma electrolytes. In view of this, two groups of animals were infused with 1.46 and 0.9 per cent NaCl respectively, at the same rate (20 ml/min) as that used for the administration of the acids. The plasma electrolyte values obtained are presented in Table 4. It is obvious that, in spite of its hypertonicity, the 1.46 per cent NaCl had but little effect on the plasma electrolytes apart from a small and transitory rise in Na^+ and Cl^- levels.

(2) *Clinical signs:* The clinical signs observed were always transitory and of a very mild nature. They consisted of mild panting with occasional twitching of the ears and tail. There seemed to be no correlation between the appearance of these symptoms and the degree of acidosis developed. Sheep K30 infused with 200 ml of 0.50 N HCl showed no signs of discomfort despite the development of an acute acidosis in which the plasma bicarbonate fell from 22.0 to 7.94 meq/l.

In addition to the above infusions, one was carried out on an abnormal wether, P5, using 0.25 N HCl. Shortly after the start of the infusion the animal showed signs of acute discomfort, then it collapsed suddenly and died after only 90 ml of the solution had been administered. On post mortem examination, death was ascribed to heart failure. This animal came from an unthrifty group of sheep which showed a tendency to develop pneumonia after dipping but always recovered

TABLE 4 Means of plasma electrolyte values (meq/l) obtained for short-term (10 min) infusions (200 ml) of 1.46 and 0.9 per cent NaCl

Solution infused	Sheep No.	Plasma electrolytes	Preinfusion sample	6 min after infusion started	2 min after infusion completed	30-40 min after infusion completed
NaCl 1.46%	K41, K30	Cl^- HCO_3^- Protein Na^+ K^+	112.99 23.13 21.10 147.66 4.68	115.04 21.51 20.87 149.43 4.70	114.83 23.01 21.03 149.84 4.70	109.36 21.48 21.10 147.38 4.66
NaCl 0.9%	K41, K51	Cl^- HCO_3^- Protein Na^+ K^+	110.25 25.60 20.08 145.59 4.53	110.43 24.96 20.10 146.78 4.58	113.59 25.00 20.21 146.00 4.50	107.13 24.87 20.00 145.34 4.49

following antibiotic therapy. The group was suspected of having originated from the Karoo where pregnancy disease is endemic. Thus despite normal pre-dosing levels of plasma electrolytes (Cl^- 103.48, HCO_3^- 26.94, Protein 23.11, Na^+ 159.26, K^+ 4.21 meq/l) Sheep P5 could have been abnormal in other respects. Nevertheless, it showed none of the clinical signs of pregnancy disease before death.

B. Long-term infusions

(1) *Plasma electrolytes and glucose:* Much difficulty was experienced with the long-term infusions of HCl. When a low concentration (0.025 N) of acid was used at slow infusion rates (1 to 4 ml/min) no acidosis could be induced. Higher concentrations of acid (0.5 to 3.6 N) in conjunction with faster (4 to 8 ml/min) or slower (1 to 2 ml/min) infusion rates also proved unsuccessful. Extensive haemolysis and haemoglobinuria occurred under these conditions resulting, in most cases, in the death of the animal. Post mortem examination revealed an extensive thrombosis of the jugular vein into which the acid was infused, and in one case, fibrinous emboli were present in both the heart and the pulmonary artery. Following several trials it was finally established that 0.05 to 0.10 N HCl infused at a rate of 4 ml per minute was capable of inducing an acidosis without producing haemolysis. The AA acid was infused into animals of 55 kg at a rate of 11.5 g/h. This rate was almost 50 per cent greater than the maximum rate of utilization of NaAA (8 g/h) for sheep found by Bergman *et al.* (1963).

The findings presented in Table 5 indicate that, following the long-term infusion of either HCl or AA acid, the most important change in plasma electrolytes

TABLE 5 Values of plasma electrolytes (meq/l) and glucose (mg%) obtained for long-term (6 to 8 h) infusions of 0.05 or 0.10 N HCL and 0.83 N AA acid in physiological saline

Acid infused	Infusion rate (ml/min)	Sheep No.	Plasma electrolytes and glucose	Preinfusion sample	Hours after infusion started			
					2	4	6	8
HCl 0.05 N	4	D7	Cl^-	107.13	108.77	109.31	108.95	—
			HCO_3^-	27.07	22.94	20.34	17.94	—
			Protein	20.35	19.99	19.65	19.99	—
			Na^+	145.67	146.73	147.00	147.38	—
			K^+	3.97	3.97	3.99	—	—
			Glucose	—	—	—	—	—
HCl 0.10 N	4	P28	Cl^-	106.43	107.53	108.49	110.01	—
			HCO_3^-	25.18	25.58	25.60	25.49	—
			Protein	20.12	20.00	19.69	19.54	—
			Na^+	—	—	—	—	—
			K^+	—	—	—	—	—
			Glucose	—	—	—	—	—
HCl 0.10 N	4	P23*	Cl^-	106.65	107.07	108.21	108.59	108.97
			HCO_3^-	26.02	22.73	19.99	18.62	17.96
			Protein	19.92	19.38	19.39	19.47	—
			Na^+	146.32	—	—	—	149.93
			K^+	4.18	—	—	—	4.03
			Glucose	84.4	85.8	82.4	74.9	62.8
HCl 0.10 N	4	P17*	Cl^-	106.37	107.00	109.65	110.98	112.12
			HCO_3^-	24.50	23.60	21.80	20.02	18.84
			Protein	20.85	21.11	21.17	20.99	20.74
			Na^+	146.73	—	149.38	—	149.25
			K^+	3.97	—	3.95	—	3.99
			Glucose	72.6	76.6	74.8	65.0	59.1
AA acid 0.83 N	2.2	K8	Cl^-	107.71	115.80	117.48	120.82	—
			HCO_3^-	27.20	23.29	22.95	17.80	—
			Protein	20.27	18.44	19.34	19.88	—
			Na^+	143.95	145.94	147.85	149.02	—
			K^+	3.89	4.02	3.80	3.78	—
			Glucose	64.1	43.9	37.1	24.0	—
AA acid 0.83 N	2.2	K48	Cl^-	106.69	107.83	109.94	112.94	—
			HCO_3^-	25.37	12.32	22.87	22.87	—
			Protein	17.06	16.35	17.81	19.29	—
			Na^+	145.62	147.97	149.98	149.85	—
			K^+	4.02	4.02	3.97	3.99	—
			Glucose	79.3	66.5	61.2	49.1	—
AA acid 0.83 N	2.2	D7	Cl^-	106.73	108.38	109.95	111.84	114.32
			HCO_3^-	26.32	24.32	20.96	18.83	16.92
			Protein	17.95	17.84	17.99	18.64	18.51
			Na^+	146.32	148.32	149.95	150.61	150.61
			K^+	4.09	4.08	3.89	3.92	3.99
			Glucose	70.0	62.5	60.1	47.3	42.9
AA acid 0.83 N	2.2	K39	Cl^-	106.32	107.32	109.99	110.87	112.47
			HCO_3^-	24.79	22.88	20.34	20.54	19.99
			Protein	20.01	19.11	19.11	20.34	20.34
			Na^+	144.58	146.10	148.23	148.35	149.98
			K^+	4.10	3.99	3.98	3.97	3.78
			Glucose	66.7	57.9	50.8	46.3	40.7

*Received protamin zinc insulin (1.8 U/kg) i.m. after the 2h blood sample was taken

was a sharp drop in bicarbonate levels. The only exception was Sheep P28 whose bicarbonate levels dropped only slightly following an infusion of 0.1 N HCl. The small rise in Na^+ and Cl^- levels is not considered to be of any significance and was probably due to the NaCl present in the solutions infused. In diabetes mellitus, electrolyte depletion usually leads to an impairment in kidney function with a resulting rise in plasma K^+ levels (Daughaday, 1960). The fact that, in the present series of experiments, K^+ concentrations remained constant, is a clear indication that the acids infused caused no renal damage to the experimental animals. The long-term infusion of AA acid caused a pronounced drop in plasma glucose of 62.6, 38.1, 38.7 and 39.0 per cent of the initial level for Wethers K8, K48, D7 and K39 respectively, although the actual levels did not fall below 24.0, 49.1, 42.9 and 40.7 mg per cent for these animals. For comparison, a fall in plasma glucose was induced in HCl-infused Wethers P23 and P17 by intramuscular injection of long-acting protamin zinc insulin (1.8 U/kg). The drop thus obtained was not so great, being only 25.6 and 18.6 per cent of the initial level, while the actual levels did not fall below 59.1 and 62.8 mg per cent.

(2) *Clinical signs:* As in the case of the short-term infusions, no clinical signs of pregnancy disease became apparent either during or following the long-term infusions, although signs of nervousness were seen on occasion. This was despite a pronounced acidosis with a concomitant fall in plasma glucose in at least six out of the eight sheep investigated. Moreover, in one of these (Sheep K8), infused with AA acid, the plasma glucose fell to a frankly hypoglycaemic level (24 mg per cent).

Discussion

The present study showed that severe acidosis with plasma bicarbonate levels as low as 7.94 meq/l could be induced in Merino wethers by short-term (10 min) or long-term (8 h) infusions of HCl, AA and/or BHB acids without the appearance of the nervous symptoms associated with ovine pregnancy disease. It would thus appear that acidosis *per se* is not immediately responsible for the induction of the nervous symptoms.

In the long-term infusions, the acidosis was accompanied by a drop in plasma glucose levels which occurred either spontaneously when AA acid was infused or artificially following administration of insulin in conjunction with infusions of HCl. The drop which occurred spontaneously (av. 44.6 per cent of the initial level) was greater than that which occurred artificially (av. 22.1 per cent). In one of the wethers (K8) infused with AA acid, the plasma glucose fell quite rapidly (6.6 mg per cent/h) to a hypoglycaemic level of 24 mg per cent. Nevertheless, no nervous signs were seen in this or any of the other animals. This was despite the fact that under similar conditions Bergman *et al.* (1963) had observed drowsiness, stupor and occasional hyperaesthesia in non-pregnant ewes infused with sodium acetoacetate at comparable infusion rates per body weight of animal. From our results it would thus seem that acidosis combined with hypoglycaemia of a few hours duration, is also not responsible for eliciting the nervous signs of pregnancy disease.

In diabetes mellitus, it is usually accepted that the nervous signs manifest themselves only after a prolonged period of uncontrolled ketosis. In ovine pregnancy disease brought on by starving preparturient ewes for 7 days, the nervous signs appeared much sooner: after only 2 days of sustained hypoglycaemic ketosis for ewes carrying twins; and after 3 days for ewes bearing single lambs (Katz & Bergman, 1966; Procos & Gilchrist, 1966). Despite the appearance of clinical signs, the animals of Katz & Bergman displayed only a very mild acidosis and small changes in plasma electrolyte levels. Nevertheless, the period of sustained hypoglycaemic ketosis preceding the onset of these signs was at least five times as long as that induced in our wethers by the long-term infusion of AA acid. It is thus possible that infusions of even longer duration would be required before nervous signs are elicited in this way. It is now becoming apparent that ketosis and the accompanying acidosis can, apart from their effect on electrolyte balance, induce a number of closely related metabolic and hormonal disturbances, at least in dogs, which could lead to nervous signs, but which take time to develop. On the other hand, the hazards associated with the intravenous infusion of inorganic acids and ketone bodies in sheep for 48 hours or longer are so great and so numerous, that it is doubtful whether nervous symptoms could ever be induced by means of this technique.

Summary

Severe acidosis was induced artificially in sheep by the infusion of hydrochloric, betahydroxybutyric and acetoacetic acids. No clinical signs, apart from slight panting, were observed and it can therefore be concluded that acidosis *per se* is not immediately responsible for the induction of the nervous signs displayed by sheep suffering from pregnancy disease.

Acknowledgements

The authors wish to thank Professor R. Clark for facilities for carrying out the early stages of this work, Dr R. C. Tustin for performing the post mortem examinations and for his expert advice on their interpretation, and Dr F. M. C. Gilchrist for reading the manuscript.

References

Bergman, E. N., Kon, K. & Katz, M. L., 1963. Quantitative measurements of acetoacetate metabolism and oxidation in sheep. *Am. J. Physiol.*, 205, 658-662.

Cameron, H. S. & Goss, H., 1940. Preparturient paresis in ewes. *J. Am. vet. med. Ass.*, 96, 165-169.

Carr, M. H. & Schloerb, F. R., 1959. Blood components in the dog: normal values. *J. Lab. clin. Med.*, 53, 646-652.

Clark, R., 1959. A study of the plasma sodium and potassium levels in normal Merino sheep. *Onderstepoort J. vet. Res.*, 28, 229-235.

Daughaday, W. H., 1960. Diabetes, Edited by R. H. Williams. New York: Harper & Row.

Katz, M. L. & Bergman, E. N., 1966. Acid-Base and electrolyte equilibrium in ovine pregnancy ketosis. *Am. J. vet. Res.*, 27, 1285-1292.

McClymont, G. L. & Setchell, B. P., 1955. Ovine pregnancy toxemia. I. Tentative identification as a lipoglycaemic encephalopathy. *Aust. vet. J.*, 31, 53-68.

McGlymont, G. L., Wynne, K. N., Briggs, P. K. & Franklin, M. C., 1967. Sodium chloride supplementation of high grain diets for fattening Merino sheep. *Aust. J. agric. Res.*, 8, 83-90.

Pierce, A. W., 1959. Studies on salt tolerance in sheep. *Aust. J. agric. Res.*, 10, 725-735.

Procos, J. & Gilchrist, F. M. C., 1966. Ovine Ketosis. V. Ketone body and blood glucose levels of Merino ewes during the preclinical, clinical and postclinical stages of pregnancy disease. *Onderstepoort J. vet. Res.*, 33, 161-167.

Sampson, J., Gonzaga, A. C. & Hayden, C. E., 1933. The ketones of the blood and urine of the cow and ewe in health and disease. *Cornell Vet.*, 23, 184-207.

Schales, O. & Schales, S. S., 1941. A simple and accurate method for the determination of chloride in biological fluids *J. biol. Chem.*, 140, 879-884.

VAN SLYKE, D. D. & NEILL, J. M., 1924. The determination of gases in blood and other solutions by vacuum extraction and manometric measurements. *J. biol. Chem.*, 61, 523-573.

WEICHSELBAUM, T. E., 1946. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am. J. clin. Path.*, 16, 40-49.

Printed in the Republic of South Africa by Heer Printing Company (Pty) Ltd., for the Government Printer, Pretoria