erythrocyte GSH were carried out using 2 inbred strains of mice and their F_1 hybrids. 1. The erythrocyte GSH level in the Z(C_3H) (alloxan resistant) strain and the F_1 hybrid was significantly higher than it was in the D_8 (alloxan susceptible) strain. 2. The Z(C_3H) strain and the F_1 hybrids were equally resistant to alloxan, whereas the D_8 strain was significantly more susceptible to alloxan. 3. Thus, both the blood GSH level and the degree of alloxan susceptibility also appears to be genetically controlled.

1. Martinez, C., Grande, F., Bittner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 236.

2. Lazarow, A., *ibid.*, 1946, v61, 441.

3. ———, *ibid.*, 1947, v66, 4.

4. Grunert, R. R., Phillips, P. H., *J. Biol. Chem.*, 1949, v181, 821.

5. Nath, M. C., Hatwalne, V. G., Gadgil, J. S., *Biochem. J.*, 1953, v53, 799.

6. Nath. M. C., Gadgil, J. S., Hatwalne, V. G., *ibid.*, 1953, v53, 481.

7. Houssay, B. A., *Am. J. Med. Sc.*, 1950, v219, 353.

8. Patterson, J. W., Lazarow, A., *Methods of Biochem. Anal.*, 1955, v2, 259.

9. Van Allen, C. M., *J. Lab. and Clin. Med.*, 1924-5, v10, 1027.

10. Folin, O., Malmros, H., *J. Biol. Chem.*, 1929, v83, 115.

11. Litarczek, G., Dinischiouti, G. T., *Compt. rend. soc. biol.*, 1933, v114, 285.

12. Dohan, J. S., Woodward, G. E., *J. Biol. Chem.*, 1939, v129, 393.

13. Dunn, J. S., Sheehan, H. L., McLetchie, N. G. B., *Lancet*, 1943, v1, 484.

14. Lazarow, A., *Physiol. Revs.*, 1949, v29, 48.

Received June 26, 1958. P.S.E.B.M., 1959, v100.

Effect of Volatile Fatty Acids, Sodium and Potassium Bicarbonate in Purified Diets for Ruminants.*† (24504)

GENNARD MATRONE, H. A. RAMSEY AND G. H. WISE
Animal Nutrition Section, Department of Animal Industry, N. C. State College, Raleigh

A previous report(1) showed that growth of lambs fed a purified diet containing a mixture of glucose and salts of acetic, propionic and butyric acid was superior to that of lambs fed diets containing a mixture of either glucose and starch or glucose, starch and cellulose. After a period of 2 years, all 3 animals on glucose-starch diet and 2 of 3 animals on cellulose diet died, whereas 5 of 6 animals on volatile acids diet survived. Further evidence for the adequacy of "acids" diet was furnished by the fact that the 5 survivors were normal in weight and in appearance. The present study was undertaken to investigate the factor(s) responsible for the superiority of volatile-fatty-acids diet. Data obtained from 2 experiments are presented.

Procedures.‡ Details of biological procedures were the same as those described previously(1) except that in this study all lambs were restricted to amounts they would consume in 3 feedings daily. Prior to placing the lambs on experiment, they were treated with *Clostridium perfringens* type D bacterin vaccine to prevent enterotoxemia. Volatile fatty acids were determined by Keeney's method (2) on samples of rumen fluid taken by stomach tube 6 hours after feeding, during latter part of first experiment. In Exp. I, diets 2, 4, 5 and 6 shown in Table I were tested. Diet 2, used as positive control, has the same formulation as the "complete" volatile acids diet used previously(1); diet 4 is similar to 2 except that Na butyrate is omitted, and diets 5 and 6 correspond to 2 and 4, respectively, except that triacetin is substituted for acetate salts. Ten lambs averaging 31 lb initially were assigned to the 4 diets; 2 lambs to each of diets 2 and 4, and 3

* Approved by Director of Research for publication as paper No. 947 in the Journal Series.

† Supported in part with grant from Herman Frasch Fn.

‡ The authors express sincere appreciation to F. A. Lane for care and feeding of animals and to Clara Bunn for technical assistance.

TABLE I. Purified Diets Used in Investigation.

Components	Diet 1	Diet 1 (Na + K)	Diet 2	Diet 4	Diet 5	Diet 5 (Na + K)	Diet 6
	%						
Casein	30	26.5	30.0	30.0	30.0	26.5	30.0
Glucose	36	31.8	24.1	27.7	27.7	24.5	24.1
Corn starch	20	17.7					
Hydrog. veg. oil	4	3.5	4.0	4.0	4.0	3.5	4.0
Na acetate			9.0	9.0			
K "			8.5	8.5			
Mg "			3.0	3.0			
Na propionate			3.0	3.0	3.0	2.7	3.0
Ca "			4.8	4.8	4.8	4.2	4.8
Na butyrate			3.6				3.6
Triacetin					20.5	18.1	20.5
$KHCO_3$		4.4				4.4	
$NaHCO_3$		7.3				7.3	
Vitamin mixture*	5	4.4	5.0	5.0	5.0	4.4	5.0
Mineral "	5†	4.4†	5.0‡	5.0‡	5.0§	4.4§	5.0§

* Vitamin mixture (5 lb): thiamine HCl, 400 mg; riboflavin, 850 mg; nicotinic acid, 1.13 g; Ca pantothenate, 1.42 g; pyridoxine HCl, 570 mg; folic acid, 57 mg; p-aminobenzoic acid, 1.13 g; inositol, 11.35 g; biotin, 11.4 mg; choline chloride, 113.45 g; menadione (2-methylnaphthoquinone), 115 mg; 0.1% B_{12} in mannitol, 4.66 g; alpha tocopherol acetate, 570 mg; glucose, 2132 g. Administered 4000 I.U. of A and 400 I.U. of D/day/100 lb body wt via capsules.

† Mineral mixture (5 lb): $CaHPO_4$, 818 g; KCl, 273 g; NaCl, 239 g; $MgSO_4$, 204 g; $CuSO_4 \cdot 5H_2O$, 893 mg; $FeSO_4 \cdot 2H_2O$, 7648 mg; $MnSO_4 \cdot H_2O$, 1399 mg; ZnO, 2263 mg; $CoCO_3$, 9 mg; KI, 6 mg; glucose, 722 g.

‡ Mineral mixture (5 lb): Cu, Fe, Mn, Zn, Co and I salts same as in (†). NH_4Cl, 138 g; H_3PO_4 (85%), 226 g; glucose, 1892 g.

§ Mineral mixture (5 lb): Cu, Fe, Mn, Zn, Co and I salts same as in (†). KCl, 273 g; $MgSO_4$, 204 g; NH_4Cl, 138 g; H_3PO_4 (85%), 226 g; glucose, 1415 g.

Grateful acknowledgment is made to Buckeye Cotton Oil Co., Cincinnati, O., for hydrogenated vegetable oil (Primex B and C); to E. I. duPont de Nemours and Co., Wilmington, Del., for Ca and Na propionate; to Union Carbide Chemical Co., Charlotte, N. C., for Na and K acetate; to R. P. Scherer Corp., Detroit, Mich., for vit. A and D; to Hoffmann-LaRoche, Nutley, N. J., for biotin; and to Merck and Co., Rahway, N. J., for other vitamins.

TABLE II. Averages of 24-Week Weight Gains and Feed Intakes of Lambs in Exp. I.

Diet No.	Triacetin	Na butyrate	Feed intake, lb	Wt gain, lb
2	0	+	141	39 (2)*
4	0	0	227	51 (2)
5	+	+	108	+2 (3)
6	+	0	105	−1 (3)

* No. in parentheses = No. of lambs receiving diet.

to each of diets 5 and 6. Exp. II was conducted in 2 parts. The first consisted of 2 replications of diets 4, 1(Na + K) and 5 (Na + K), the latter 2 diets being identical to diets 1, described previously(1), and 5, respectively, except for inclusion of Na and K bicarbonates. The second part involved 2 more replications of the diets in part I and 2 replications of diets 1 and 5 (Table I). Sixteen lambs averaging 27 lb were used.

Results. Lambs fed triacetin-containing diets, 5 and 6, gained little or none during 24 weeks, indicating that triacetin was not a satisfactory substitute for acetate salts (Table II). The subsequent death of 2 lambs in each group gave further evidence of the inadequacy of these diets. Lambs on diet 4 consumed more and gained more than lambs on diet 2, but feed efficiencies were similar, *e.g.*, 4.45 and 4.65 lb of feed/lb of gain, respectively. Percentage distribution of ruminal volatile acids was similar for all diets (Table III). Comparison of C_4 acid fractions shows that butyrate was present in rumen

TABLE III. Mean Fatty Acid Composition of Rumen Fluid from Lambs in Exp. I.

Diet No.	Samples analyzed	Acid fractions, molar % of total C_2	C_3	C_4	C_5	C_6 and higher
2	9	52.5	21.1	12.4	5.0	8.9
4	8	49.1	23.4	12 8	6.3	8.4
5	8	46.7	23.4	14.0	7.1	8.8
6	5	45.2	23.4	16.6	7.3	7.6

TABLE IV. Averages of Weight Gains and Feed Intakes of Lambs in Experiment II.*

Diet No.	Part 1 Feed intake, lb	Part 1 Gain, lb	Part 2 Feed intake, lb	Part 2 Gain, lb	Avg, parts 1 + 2 Gain, lb/wk
1			41	-1.0 (1)‡	
1 (Na + K)	159	39.5 (2)†	69	13.8 (2)	1.9 (4)
4	151	36.3 (2)	60	11.5 (2)	1.7 (4)
5			32	-1.3 (2)	
5 (Na + K)	123	27.0 (2)	71	17.5 (2)	1.7 (4)

* Part 1 covers period of 16 wk and Part 2 a period of 10 wk.
† No. in parentheses = No. of lambs receiving diet.
‡ One of the two animals on diet 1 died after 5 wk on experiment.

samples in about the same ratio irrespective of presence of butyrate in the diet.

These findings agree with observations from a supplementary study with mature sheep and calves fed purified diets either without dietary voltaile acids or with a supplement of either propionate alone or acetate alone. Irrespective of diet the acid fractions shown in Table III were always present, and ratios of C_5 and lower fractions were remarkably similar among different diets.

The results of Exp. II, Table IV, show that the over-all gains of lambs fed diets 1 (Na + K) and 5 (Na + K) are equal to those fed control diet 4. Feed efficiencies were also similar, *e.g.*, 4.08, 4.43 and 4.37 lb feed/lb gain for diets 1(Na + K), 4, and 5 (Na + K), respectively. The poor performance of animals fed diets 1 and 5 in part 2 confirms earlier results(1) and furnishes additional evidence showing the beneficial effect on growth of adding Na and K bicarbonates to the diets.

Discussion. The results of the first experiment showing similar patterns of ruminal fatty acids irrespective of diets fed substantiate reports(3,5,6) indicating that volatile fatty acids are formed in the rumen from a variety of substrates. These data probably account for the finding that dietary volatile fatty acids were not the limiting factor of the purified diets investigated. Our results suggest that bulk or roughage is not an essential in the diet of ruminants and that a purified diet devised for simple-stomach animals is suitable for ruminants provided it is adequately supplemented with Na and K cations in the form of bicarbonate. The role of these cations in the purified diets is most probably associated with the buffering capacity in the rumen.

With a normal roughage diet, as volatile acids are formed, they are neutralized by buffers of the saliva, which is high in Na bicarbonate(4). Mineral cations of saliva entering the rumen are reabsorbed maintaining the alkaline reserve of blood and becoming again available for recirculation by saliva. Thus, a fixed amount of buffer can be used to neutralize a much larger amount of acid. McDougall(4) estimated that the CO_2 content of saliva is about 4 times the normal alkali reserves of sheep's blood and cited Zuntz's observation that amount of alkali secreted each day in the saliva of oxen was about 6 times that contained in blood. Purified diets fed here consist of readily fermentable substances, and therefore, when fed to the ruminant, the ruminal acids would be formed more quickly than on a normal diet(3), thus requiring more buffering capacity at a given time. Furthermore, since animals fed purified diets did not ruminate appreciably, flow of saliva was probably reduced markedly(4), thereby decreasing ruminal buffering capacity of the animal. Under these circumstances, the appetite of the lambs is depressed by an adaptive mechanism to maintain a physiologically compatible environment in the rumen. Conceivably the role these buffers play in the ruminal environment also effects shifts in population of rumen microorganisms and in quantities and rate of transport of end products which in turn affect productive energy of the diet.

Summary. Two experiments with lambs were conducted to determine the dietary essentials supplied by various purified diets. In

the first experiment triacetin could not replace acetate salts in the diet and butyrate could be omitted. Butyrate was found present in the same amount in the rumen contents irrespective of its presence in the diet. The second experiment showed growth could be obtained on simplified diets supplying energy as carbohydrate or triacetin if sodium and/or potassium were added to the ration.

1. Matrone, G., Ramsey, H. A., Wise, G. H., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 731.

2. Keeney, M., Misc. publ. No. 238, 1955, p23, Maryland Agric. Exp. Sta.

3. Phillipson, A. T., McAnally, R. A., *J. Exp. Biol.*, 1942, v19, 199.

4. McDougall, E. I., *Biochem. J.*, 1948, v43, 99.

5. Elsden, S. R., *J. Exp. Biol.*, 1945, v22, 51.

6. Johns, A. T., *N. Z. J. Sci. Tech.*, 1953, v35, 262.

Received July 24, 1958. P.S.E.B.M., 1959, v100.

Role of Sodium in Production of Myocardial Necroses by Stress.* (24505)

H. SELYE AND E. BAJUSZ

Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada

Previous experiments have shown that, following prolonged pretreatment with large doses of certain corticoids, *e.g.*, 2α-methyl-9α-chlorocortisol (Me-Cl-COL), a brief period of neuromuscular effort (induced by forced restraint) can elicit acute, massive myocardial necroses in the rat(1). Subsequent investigations revealed that the precipitating effect of neuromuscular effort is not specific, since a variety of other stressors (hot or cold baths, surgical trauma, adrenaline) also produce myocardial necroses after conditioning with Me-Cl-COL. It was found, furthermore, that certain sodium salts (phosphates, sulfates, perchlorate) can, while others (chloride, acetate, citrate, lactate) cannot replace the stressors in precipitation of myocardial necroses after Me-Cl-COL conditioning(2). No salts of cations other than Na were effective, but these observations show that the anion also plays an important role in the pathogenesis of this type of myocardial necrosis. We were particularly impressed by the fact that, although NaCl and the organic Na-salts were almost always totally ineffective after Me-Cl-COL-pretreatment, they occasionally produced very pronounced cardiac necroses, in rats damaged through fortuitous circumstances. It was, therefore, postulated that, after suitable sensitization with corticoids, certain Na-salts (phosphates, sulfates, perchlorate) produce cardiac necroses under ordinary conditions, while others (chloride and several organic salts) are inactive in themselves, but become highly effective during stress. We now wish to report upon experiments which substantiate this concept.

Materials and methods. Two hundred female Sprague-Dawley rats, with mean initial body weight of 100 g (range: 95-110 g), were subdivided into 20 equal groups, as indicated in Table I. Me-Cl-COL† was administered subcutaneously, in the form of its acetate, as a microcrystal suspension of 100 μg in 0.2 ml of water, once daily. All the Na-salts were given by stomach tube, at the dose of 2 mM, in 2 ml of water, twice daily. Neuromuscular stress was induced by maintaining the rats on a board with adhesive tape, for a period of 17 hours, on the fifth day of the experiment. The animals of all groups were killed with chloroform on the sixth day. The hearts were fixed in neutral formalin and embedded in paraffin for subsequent staining with our fuchsin technic(2). The intensity of the necroses was assessed in terms of an arbitrary scale of 0-3, and the means of these readings (with standard errors) are listed in Table I.

Results. It is evident from our data that, in these rats, cardiac necroses do not occur

* Supported by grants from Gustavus and Louise Pfeiffer Research Fn. and from Geigy Pharmaceuticals.

† Generously supplied by Upjohn Co.