lections must be made and characterized, and, as soon as possible, newly acquired strains must be frozen and stored to prevent genetic deterioration. Ideally, reservoirs of cultures should be maintained in several locations. Technical problems still remain, however. The fraction of viable cells recovered is still low, and the procedure appropriate for syngen 1 may not be applicable to other syngens.

ELLEN M. SIMON
Department of Zoology, University of Illinois, Urbana 61801
SHUH-WEI HWANG
American Type Culture Collection, Rockville, Maryland 20852

References and Notes

1. D. L. Nanney, *J. Protozool.* **6**, 171 (1959); ——— and M. J. Nagel, *ibid.* **11**, 465 (1964).
2. S. L. Allen, *ibid.* **10**, 413 (1963); *ibid.*, in press; D. L. Nanney, *Genetics* **48**, 737 (1963).
3. J. O. Corliss, *Trans. Amer. Microscop. Soc.* **71**, 159 (1952); *Parasitology* **43**, 49 (1953); D. F. Gruchy, *J. Protozool.* **2**, 178 (1955).
4. S. Hwang, E. E. Davis, M. T. Alexander, *Science* **144**, 64 (1964); G. T. Wang and W. C. Marquardt, *J. Protozool.* **13**, 123 (1966).
5. T. M. Sonneborn, in *The Species Problem*, E. Mayr, Ed. (AAAS, Washington, D.C., 1957), p. 155. Each taxonomic "species" of ciliated protozoans that has been adequately studied includes several sexually isolated varieties called syngens.
6. Prototype, Canal Industrial Corp., Rockville, Maryland.
7. The work at Illinois was carried out under a PHS grant GM-07779 to D. L. Nanney. The work at ATCC was supported by NSF grant GB-2946 to Shuh-wei Hwang.

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Siliceous Urinary Calculi in Calves: Prevention by Addition of Sodium Chloride to the Diet

Abstract. *Urinary calculi were found in all but one of 14 calves given a ration associated with the formation of siliceous calculi. No calculi were found in a similar group of 14 calves given the same ration with sodium chloride added (4 percent). It is suggested that sodium chloride prevented calculus formation by increasing water intake and urine volume, with a consequent reduction in the concentration of silicic acid in the urine.*

Urinary calculi composed largely of amorphous silica occur frequently in range cattle in the northern Great Plains of North America (*1, 2*). Obstruction of the urethra is a major cause of death among cattle in this area. During the fall and winter, when the incidence of urethral obstruction is highest, the silica content of the native prairie grasses is often higher than during spring and early summer (2, 3). In most of the grass species, however, the content of silica rarely falls below 2 percent at any time. The concentration of silicic acid in the urine of ruminants that consume mature prairie grass is often two to three times the concentration of a saturated solution (about 19 mg/100 ml at body temperature) and markedly higher than the concentration in urine when leguminous forages are consumed (*4*). The higher concentration in the urine of animals that consume prairie grass is due both to a lower urine production and a higher rate of silicic acid excretion.

Silicic acid polymerizes in simple aqueous solution when the concentration exceeds the saturation value (*5*). The rate of polymerization increases exponentially with increase in silicic acid concentration (*5*); it also increases with rising temperature and in the presence of electrolytes (*5*). Both the high concentration of silicic acid and the presence of electrolytes in the urine of ruminants that consume prairie hay would favor rapid polymerization of the silicic acid. The evidence suggests, on the other hand, that polymerization does not occur in urine when the concentration of silicic acid is below saturation (*6*). Since polymerization of silicic acid is involved in the formation of calculi, it is reasonable to assume that calculi will not form in cattle that are consuming prairie grass as long as the concentration of silicic acid in the urine is kept below 19 mg/100 ml. The experiment reported here supports this assumption. Calculus formation was prevented in calves receiving a prairie-grass hay diet supplemented with enough sodium chloride to increase the urine volume and maintain the silicic acid concentration below the saturation value.

Each member of two groups of 14 steer calves, 10 Holstein and 4 Jersey, was given pelleted prairie grass hay as desired from birth to 10 months of age. The predominant grass in the pellets was *Festuca scabrella* Torr. The pellets given to one group contained 4 percent sodium chloride but those given to the other group contained none. For the first 5 months, the Holsteins received 4.5 kg of milk per day and the Jerseys received 2.7 kg. Water was always available to both groups. The prairie hay contained 6.5 percent protein, 10.4 percent ash, 6.3 percent silica, and 4.1 percent nonsilica ash.

Water intake was measured during the last 2 weeks of the experiment, and on 2 consecutive days during this period urine samples were obtained and analyzed for silicic acid (*7*). At the end of the experiment all calves were killed, and their kidneys and bladders were examined for calculi. Calculi in the bladder were obtained by washing the contents with running water into an 80-mesh sieve and in the kidneys by opening and washing each calyx. The calculi were air-dried, weighed, and pooled for silica analysis (*7*).

The mean water intake of the animals given salt was higher than in those not given salt (Table 1). Since variations within groups in feed intake were rather large, and water intake is related to feed intake, values for water intake per unit of feed intake are also given in Table 1. There were highly significant differences between groups with respect to the mean silicic acid concentration in the urine. The concentration of silicic acid in the urine of all calves that received the salt supplement was below saturation; the con-

Table 1. Growth rate, water intake, urinary silicic acid concentration, and weight of calculi in kidneys and bladders of calves receiving prairie hay or prairie hay plus 4 percent sodium chloride. Results are given as means and standard errors.

Growth rate (kg/day)	Water intake (kg/day)	Water intake/feed intake (kg/kg)	Urinary silicic acid (mg/100 ml)	Calculi in kidneys and bladder (mg)
		Prairie hay		
0.76 ± 0.04	19.4 ± 1.37	2.74 ± 0.11	27.2 ± 1.64	49.4 ± 15.0
		Prairie hay plus 4 percent NaCl		
0.71 ± 0.04	29.1 ± 2.21	3.67 ± 0.09	14.8 ± 0.46	0
		Probability		
>.200	<.005	<.001	<.001	<.01

centration of silicic acid in the urine of all but one of the calves not receiving the supplement was above saturation.

There were calculi both in the kidneys and bladders of all but one of the calves that did not receive salt, but there were none in those of calves that did receive salt. The silica content of calculi in the bladder was 74.0 percent and of those in the kidney 67.4 percent. The presence of the salt in the ration did not have a significant effect on the growth of the calves.

There were large variations in the total amounts of calculi present in the calves in the group that did not receive salt (range 0 to 188 mg). Four of these calves had more calculus material in the bladder than the average amount (about 50 mg) contained in stones that had previously caused urethral obstruction in ten similar calves. The amounts of calculi present were not related to the concentration of silicic acid in the urine. Therefore, other factors must also have affected the amounts of calculi formed. These factors are of considerable practical significance because they determine which animals are likely to develop urethral obstruction when on a diet of prairie grass.

It is suggested that sodium chloride prevented the formation of siliceous calculi in cattle given prairie grass hay by increasing water intake and urine volume and consequently reducing the concentration of silicic acid in the urine. Whether the administration of water to cattle in amounts greater than those they normally drink when consuming prairie hay will also prevent calculus formation remains to be determined.

C. B. BAILEY

Research Station, Canada Department of Agriculture, Lethbridge, Alberta

References and Notes

1. R. F. Connell, F. Whiting, S. A. Forman, *Can. J. Comp. Med. Vet. Sci.* **23**, 41 (1959).
2. K. G. Parker, *J. Range Management* **10**, 105 (1957).
3. L. Bezeau, A. Johnston, S. Smoliak, *Can. J. Animal Sci.* **46**, 625 (1966).
4. C. B. Bailey, *Am. J. Vet. Res.*, in press; S. A. Forman and F. Sauer, *Can. J. Animal Sci.* **42**, 9 (1962).
5. H. K. Iler, *The Colloid Chemistry of Silica and Silicates* (Cornell Univ. Press, Ithaca, 1955); K. B. Krauskopf, in *Silica in Sediments*, H. A. Ireland, Ed. (Special Publ. No. 7, Society of Economic Paleontologists and Mineralogists, Tulsa, Oklahoma, 1959).
6. L. H. P. Jones and K. A. Handreck, *J. Agr. Sci.* **65**, 129 (1965); F. Sauer, D. H. Laughland, W. M. Davidson, *Can. J. Biochem. Physiol.* **37**, 183 (1959).
7. E. J. King, B. D. Stacy, P. F. Holt, D. M. Yates, D. Pickles, *Analyst* **80**, 441 (1955).

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Protein Subunits: A Table

Table 1 is a list of proteins in which subunits are held together by noncovalent bonds. The entries are listed approximately in the order of increasing molecular weight of the macromolecular unit normally isolated from its natural source.

Individual polypeptide chains, held together by disulfide linkages, have not been individually classified as subunits; in insulin, for example, the subunit weight listed is 6000, that of the A and B chains together, since these chains are linked by disulfide bonds.

In many instances the subunit listed may not be the minimal subunit obtainable. The entry listed in Table 1 contains the minimal subunit that has been unequivocally obtained under environmental conditions that raise no suspicion of cleavage of peptide bonds. For some proteins, two (or more) stages

Table 1. Subunit constitution of proteins. Parentheses indicate doubt.

Protein	Molecular weight	Subunits	
		No.	Molecular weight
Insulin (*2*)	11,466	2	5,733
Thrombin (*3*)	31,000	(3)	(10,000)
β-Lactoglobulin (*4*)	35,000	2	17,500
Avidin (*5*)	53,000	3	18,000
Hemoglobin (*6*)	64,500	4	16,000
Glycerol 1-phosphate dehydrogenase (*7, 8*)	78,000	2	40,000
Alkaline phosphatase (*9*)	80,000	2	40,000
Enolase (*10*)	82,000	2	41,000
Liver alcohol dehydrogenase (*11*)	84,000	2	42,000
Procarboxypeptidase (*12*)	87,000	1	34,500
		2	25,000
Firefly luciferase (*13*)	92,000	2	52,000
Hexokinase (*14*)	96,000	4	24,000
Hemerythrin (*15*)	107,000	8	13,500
Tryptophan synthetase A (*16*)	29,000	1	29,000
Tryptophan synthetase B (*16*)	117,000	2	60,000
Mammary glucose 6-phosphate dehydrogenase (*17*)	130,000	2	63,000
Glyceraldehyde 3-phosphate dehydrogenase (*8, 18*)	140,000	4	37,000
Aldolase (*19*)	142,000	3	50,000
Lactic dehydrogenase (*20*)	150,000	4	35,000
Yeast alcohol dehydrogenase (*7, 21*)	150,000	4	37,000
Ceruloplasmin (*22*)	151,000	8	18,000
Threonine deaminase (*23*)	160,000	4	40,000
Thetin homocysteine methylpherase (*24*)	180,000	3–4	50,000
Fumarase (*25*)	194,000	4	48,500
Serum lipoprotein (*26*)	200,000	6	36,500
Tryptophanase (*27*)	220,000	2	(125,000)
Pyruvate kinase (*28*)	237,000	4	57,200
Catalase (*29*)	250,000	4	60,000
Phycocyanin (*30*)	266,000	2	134,000
	134,000	3	46,000
Mitochondrial adenosine triphosphatase (*31*)	284,000	10	26,000
Aspartyl transcarbamylase (*32*)	310,000	2	96,000
		4	30,000
Lipovitellin (*33*)	400,000	2	200,000
Apoferritin (*34*)	480,000	20	24,000
Urease (*35*)	483,000	6	83,000
Phosphorylase (*36*)	495,000	4	125,000
Fraction I protein, carboxydismutase (*37*)	515,000	24	22,000
β-Galactosidase (*38*)	520,000	4	130,000
	130,000	3–4	(40,000)
Myosin (*39*)	620,000	3	200,000
Pyruvate carboxylase (*40*)	660,000	4	165,000
	165,000	4	45,000
Thyroglobulin (*41*)	669,000	2	335,000
Propionyl carboxylase (*42*)	700,000	4	175,000
Lipoic reductase-transacetylase (*43*)	1,600 000	60	27,000
Glutamic dehydrogenase (*44*)	2,000,000	8	250,000
	250,000	5	50,000
Hemocyanin (*45*)	300,000–9,000,000		385,000
			70,000
			35,000
Chlorocruorin (*46*)	2,750,000	12	250,000
Bromegrass mosaic virus (*47*)	4,600,000	180	20,000
Turnip-yellow mosaic virus (*48*)	5,000,000	150	21,000
Poliomyelitis virus (*49*)	5,500,000	130	27,000
Cucumber mosaic virus (*50*)	6,000,000	185	21,500
Alfalfa mosaic virus (*51*)	7,400,000	160	35,000
Bushy stunt virus (*52*)	9,000,000	120	60,000
Potato virus X (*53*)	35,000,000	650	52,000
Tobacco mosaic virus (*54*)	40,000,000	2130	17,500