They were then placed on a caries-producing diet,[2] ad libitum, for 100 days, after which they were sacrificed and their caries scored and recorded by the method of Keyes (*3*). The control group (14 ♀ and 14 ♂) received the caries-producing diet only, and the ingestion group (13 ♀ and 12 ♂) received the same diet plus dibasic ammonium phosphate 1% and urea 0.6%—the ratio commonly found in ammoniated dentifrices.

TABLE 1

	Caries scores				
	No. of animals	Mean caries score	Standard deviation	t	p
Female					
control	14	26.4	40.0	2.11*	.05
ingestion	13	1.9	5.2		
Littermates					
control	7	36.8	46.6	1.93†	.10
ingestion					
Male					
control	14	57.5	76.3	2.03*	.05
ingestion	12	10.0	16.4		
Littermates					
control	9	66.1	83.4	2.24†	.06
ingestion					
Male and female groups combined					
control	28	42.0	62.9	2.77*	.006
ingestion	25	5.8	12.6		
Littermates					
control	16	53.3	71.2	2.90†	.011
ingestion					

$$* \; t = \frac{\bar{x} - \bar{y}}{SE_{\bar{x}-\bar{y}}} \qquad \text{where } SE_{\bar{y}-\bar{x}} = \sqrt{\frac{\hat{\sigma}^2}{N_x} + \frac{\hat{\sigma}^2}{N_y}}$$

$$\text{where } \hat{\sigma}^2 = \frac{N_x s_x^2 + N_y s_y^2}{N_x + N_y - 2}$$

$$\dagger \; t = \frac{\bar{d}}{\frac{s_d}{\sqrt{N-1}}}$$

From the mean caries score in Table 1, it appears that a striking inhibition of caries has occurred. The variation is considerable, however, and, in general, more than reported in other studies. It is possible that, if the animals had continued longer on the diet, the scores would have been higher and possibly more homogeneous. We are assuming a 1% level of significance when testing the difference between two means because of the small number of animals in the groups, and the relative infrequency of valid statistical evaluation of previously published experimental caries data. In addition, one must keep in mind the possibility that the effect of ingestion of these compounds by hamsters may not be comparable to the effect produced in human beings by these compounds.

Previous reports generally have indicated a sex difference in caries activity, if the animals are maintained for approximately 100 days on a caries-producing diet, although the difference is less striking when the animals are maintained for longer periods of time. It is of interest to combine the male and female animals in the control and ingestion groups. With more animals in each group, the difference between the mean caries scores seems more significant (i.e., below the .01 level). These data strongly indicate that the animals employed enjoyed a reduction in caries experience associated with the ingestion of the ammoniated products studied.

[2] Whole wheat flour, 30%; whole powdered milk, 30%; cornstarch, 20%; confectioners' sugar, 15%; alfalfa meal, 4%; and sodium chloride, 1%.

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Chloromycetin in the Treatment of "Red Leg"[1]

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The epidemic disease "red leg" has long been a source of difficulty to workers using any of the usual species of frogs as experimental animals. Control of the disease has always been unsatisfactory, as far as can be ascertained from the literature. It was therefore considered worth while to report the use of chloromycetin (chloramphenicol) in the successful treatment of the homologue of frog red leg in a toad, *Bufo marinus.*

The disease was apparently first described by Ernst (*6*), who isolated a bacterium, which he named *Bacillus ranicida,* from infected frogs. Sanarelli (*9*) studied an organism, which he isolated from frogs with red leg and named it *B. hydrophilus fuscus.* He reported the organism to be pathogenic for a variety of anurans and urodeles and briefly described some of the pathological findings in infected frogs. Russell (*8*) reported the pathology of the infection in more detail and described the experimental infectivity of the organism for some of the common laboratory mammals when injected intravenously; he also mentioned the production by the organism of at least two toxins that had pronounced effects upon frog cardiac and skeletal muscle and central nervous system. The nomenclature was revised to *Proteus hydrophilus* in the fifth edition of Bergey's *Manual* (*1*) in 1939. In the sixth edition (*3*) it was changed to *Pseudomonas hydrophila.* In 1942 Kulp and Borden (*7*) made an extensive study of the bacteriology of the disease, describing two distinct strains of the organism from different sources and reviewing the pathology in frogs. They indicated the existence of "carrier" frogs that harbor the organism in their gall bladders.

The disease in anurans is septicemic and is remarkable

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for its virulence and fulminating course. All organ systems are involved, more or less, by the occurrence of widespread petechial hemorrhages and/or septic thrombi, as well as disseminated miliary abscesses. In frogs the skin of the legs becomes markedly hyperemic and hemorrhagic. Death is the usual termination of the disease.

During the course of studies in this laboratory on *Bufo marinus*, three specimens of native toads (*B. boreas* and *B. woodhousii*) were introduced into a colony of 30 specimens of *B. marinus* that had been in excellent health in the laboratory for 16 months. Within 10 days the first case of an epidemic disease, which destroyed a third of the colony before it was controlled, appeared. The disease was studied bacteriologically[2] and pathologically and found to be a classical example of *F. hydrophila* septicemia. Source of the infection was subsequently traced to an infected frog colony with which the native toads had been housed prior to their acquisition by the author.

In view of the reports that chloromycetin (chloramphenicol) is effective *in vitro* (*2*) against various species of *Proteus* and *Pseudomonas* and in the treatment of human urinary tract infections (*4*), this drug was tried on the remaining animals in the colony. At the beginning of treatment most of the animals showed the early stigmata of the disease, namely, moderate reddening and capillary dilatation over the lower belly. The dosage was derived empirically from that used in man and other mammals, and set at 5 mg/100 g initially, followed by 3 mg/100 g twice daily for five days. The drug was dissolved in distilled water in concentrations of 5 mg/ml for the initial dose and 3 mg/ml for subsequent doses. Solution of these amounts was readily accomplished (in contradiction to the solubility data given by Woodward [*10*] by slight warming. The solutions were administered by gastric intubation, a procedure which is easily performed in these animals.

One of the treated animals was in an advanced stage of the disease (signs of extensive central nervous system damage) at the beginning of therapy. This animal survived 4 more days (twice the survival expectancy of untreated animals in advanced stages) but succumbed to what superficially resembled myocardial decompensation in mammals, presumably due to irreversible myocardial damage incurred before inception of therapy. The remainder of the animals responded well. Now, some two months later, they have shown no further evidence of the disease and again are in their former state of excellent health.

These results suggest that colonies of amphibians may be maintained free from infection by *P. hydrophila* by the administration of chloromycetin at the time the colony is established, either in the water in which the animals are kept, or, preferably, in the manner described above. Similar measures might be practiced on new additions to the colony. The latter seems desirable in view of the report by Emerson and Norris (*5*) that the disease is endemic in many of the natural habitats from which frogs are collected commercially.

[2] Thanks are due Elizabeth O'Toole, of the Department of Bacteriology of the University of Colorado School of Medicine, with whom a detailed report of the pathology and bacteriology of the infection in *B. marinus* will be published elsewhere.

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A Diploid Form of *Medicago sativa* L.

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There have been numerous cytological investigations of common alfalfa, *Medicago sativa* L. Reviews by Fryer (*2*) and Senn (*4*) show that the normal chromosome number in somatic tissues is 32. Fryer reported one exceptional plant as having 35 chromosomes. Other abnormal numbers were 31 and 33, found by Skovsted (*5*) in a study of twin seedlings. The work of Ledingham (*3*) strongly suggests that the species originated as an autotetraploid, and Tysdal *et al.* (*7*) have concluded that at least certain genetic data could be interpreted on the basis of tetrasomic ratios rather than the commonly used disomic approach. If *M. sativa* has an autotetraploid origin, it is to be expected that fertile 16-chromosome forms would exist. So far as the authors are aware, no such forms have been reported. It is the purpose of this paper to record what appears to be a 16-chromosome form of *M. sativa* and to present preliminary data on its cytology and breeding behavior.

In 1947 a small sample of seed labeled *Medicago sativa* was received from Russia through H. A. Senn, senior botanist, Division of Botany and Plant Pathology, Science Service, Ottawa. It was given the Saskatoon accession number S-2128. According to information accompanying the sample, it came from the Botanical Gardens, Academy of Sciences, Armenian S.S.R. at Erevan, Kanaku, U.S.S.R., and was collected from one wild-growing plant. Forty-three seedlings were established in the field nursery at Saskatoon in June, 1949.

The plants started to flower in early August, and it was then apparent that S-2128 was not a normal form of *M. sativa*. It was similar to the latter in having purple flower color, with no trace of yellow present. It was similar also in that a later examination showed the pods to have up to 3 or 4 coils and the relative lack of pubescence common to *M. sativa*. In various characters, how-