of all indicated specimens at a lower as well as at the higher temperature and carry out the procedure as follows:

We apply our di-nitro-salicylic acid method to all urines. This step eliminates the non-reducing urines which are reported sugar-negative and also provides the total reduction value of whatever reducing substances a specimen may happen to contain.

To all the reducing urines we then apply an improved (unpublished) phenyl-hydrazine test. This step eliminates all the non-sugar-reducing urines, which are also reported negative for sugar.

If phenyl-osazone crystals are present, another di-nitro-salicylic acid test is run at the lower temperature on a sample which has been adjusted so that concentrations of sugar are always 0.4%. This adjustment is readily accomplished by increasing the amount of or diluting the urine, as may be indicated by the known total reduction value of the sample. A simple calculation then gives the desired qualitative and quantitative information.

It will be observed that this simple addition to routine technics need be applied only to those specimens which are definitely known to contain some kind of sugar. It may also be noted that the actual manipulations of the new step take about 2 minutes, exclusive of time in the water bath, and that the complete procedure requires less than 2 cc. of urine.

7706

Absence of Dietary Anti-Anemia Substance in the Diet Causative of Canine Black Tongue.

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Goldberger[1] showed that autoclaved yeast possessed the power of preventing black tongue in dogs, and later that the liver fraction of Cohn and Minot,[2] which was therapeutically effective in pernicious anemia, was also prophylactic against black tongue. Autoclaved yeast was found by Strauss and Castle[3] to be capable of effecting remissions in pernicious anemia after it had been incubated with normal human gastric juice. The therapeutic similarity between

[1] Goldberger, J., and Sebrell, W. H., *Pub. Health Rep.*, 1930, **45**, 3064.

[2] Cohn, E. S., McMeekin, T. L., and Minot, G. R., *J. Biol. Chem.*, 1930, **87**, 49.

[3] Strauss, M. B., and Castle, W. B., *New England J. Med.*, 1932, **207**, 55.

canine black tongue and pernicious anemia suggested that the effect of the diet producing black tongue was due to a lack of that dietary constituent which is required for normal hematopoiesis in the human being. It has been found possible to test and substantiate this hypothesis by experiments on guinea pigs fed a diet which is causative of black tongue in the dog.

Adult guinea pigs weighing from 400 to 600 gm. were used. The animals were kept under uniform conditions with shavings as bedding. The Goldberger black tongue-producing diet was fed daily, and the animals were allowed to eat as much as they desired. The diet was composed of the following ingredients:

White corn meal,	6,000 gm.	Cotton seed oil	450 cc.
California black-eyed peas,	750 "	Calcium carbonate,	45 gm.
Casein (leached),	900 "	Sodium chloride	15 "
Cod liver oil,	225 cc.		

In addition to the special diet, each experimental animal was fed daily 2 cc. of fresh orange juice to ensure an adequate intake of vitamin C. The materials which were tested for their value in preventing the effect of the diet feeding were mixed with water and fed daily with a pipette.

TABLE I.

Exp. No.	Therapy	No. animals	Aver. wt. beginning of exp. gm.	Aver. wt. end of exp. gm.	Aver. change per animal gm.	Result
1	Controls Diet alone	5	450	325	—125	Died during 2nd and 3rd weeks
2	Liver extract Lilly No. 343 0.6 gm. q. d.	5	415	455	+40	Survived
3	Vegex 1 gm. q. d.	4	457	366	—91	Died during 2nd and 3rd weeks
4	Vegex 2 gm. q. d.	3	393	426	+33	Survived
5	Ventriculin 1 gm. q. d.	4	408	310	—98	Died 2nd-5th week
6	Ventriculin 2 gm. q. d.	3	393	268	—125	Died during 3rd week
7	Vegex 1 gm. + Ventriculin 1 gm. q. d.	4	388	425	+37	Survived

The results are presented in Table I. All of the animals fed only the vitamin C supplemented diet producing black tongue lost weight rapidly and died before the end of the third week. When a daily supplement of 0.6 gm. of liver extract No. 343 was administered, all the animals survived and showed a gain in their average weight.

A commercial yeast extract sold under the name of Vegex was found to be ineffective when 1 gm. daily was fed, but was prophylactic when 2 gm. daily were used. This same extract was employed by Strauss and Castle[3] as a source of the dietary anti-anemia factor. Desiccated hog stomach given in 2 gm. amounts daily as Ventriculin was found to be ineffective, as would be expected if it contained only the gastric without the dietary anti-anemia factor. If 1 gm. of Ventriculin plus 1 gm. of Vegex were administered, however, complete prophylaxis occurred, although neither substance was effective alone in the dosage given. The results suggest that the deficiency which is causative of canine black tongue is closely allied to the deficiency which is etiologic in pernicious anemia. Moreover, the death or survival of the guinea pig fed the diet producing black tongue may serve as a useful test for evaluating the potency of various substances used in the treatment of pernicious anemia in the human being.

Conclusion. Guinea pigs fed a diet causative of canine black tongue lose weight rapidly and die within a short period. This effect may be prevented by substances which are capable of causing remissions in pernicious anemia.

7707 C

Multiplication of Equine Encephalomyelitis Virus in Mosquitoes.

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While Davis, Frobisher and Lloyd[1] failed to find evidence of multiplication of yellow fever virus in infected *Aëdes aegypti,* the experiments of St. John, Simmons and Reynolds[2] and of Holt and Kintner,[3] though limited in number, suggest that the dengue fever virus does multiply in its transmitting insect host. We have reported[4] that *Aëdes sollicitans* fed on animals infected with the

[1] Davis, N. C., Frobisher, M., Jr., and Lloyd, W., *J. Exp. Med.*, 1933, **58**, 211.

[2] St. John, J. H., Simmons, J. S., and Reynolds, F. H. K., *Am. J. Trop. Med.*, 1930, **10**, 23.

[3] Holt, R. L., and Kintner, J. H., *Phil. J. Sci.*, 1931, **46**, 593.

[4] Merrill, M. H., Lacaillade, C. W., Jr., and Ten Broeck, C., *Science*, 1934, **80**, 251.