tein content of the diet. This relationship existed both in *ad libitum* and controlled feeding experiments. The hepatic stores of folic acid, biotin, pantothenic acid, and riboflavin found in rats fed the lower levels of protein were indicative of either frank or incipient deficiencies.

3. Leucopenia and agranulocytosis were induced readily by diets low in protein. Under these conditions the existence of the dyscrasia was correlated with the reduced fecal elimination and hepatic storage of folic acid. When diets more nearly optimal with respect to casein level, but in greatly restricted amounts, were fed, leucopenia and agranulocytosis were encountered despite the existence in many of the animals of adequate stores of folic acid. Reasons were presented for attributing the condition to the ingestion of insufficient protein and, more specifically, of certain essential amino acids.

4. Low hematocrits, hemoglobin values, and red cell counts were observed occasionally in rats ingesting low protein diets or *highly purified* diets containing succinylsulfathiazole (SST).

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pH Stability of St. Louis Encephalitis Virus.*

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Studies on several viruses have indicated that for each virus there is a range of hydrogen ion concentration over which the virus is stable and that at higher or lower pH values the virus activity is destroyed.[1-6] The observation[6] that Japanese B encephalitis virus is most stable at a hydrogen ion concentration near pH 8.5, while the other viruses so far studied were more stable in a less alkaline medium, suggested that the effect of hydrogen ion concentration on St. Louis encephalitis virus (S.L.E.) be studied to determine whether the pH at which the latter virus is stable is similar to that reported for the virus of Japanese B encephalitis. The purpose of the present communication is to report the experiments conducted to determine the hydrogen ion concentration at which St. Louis encephalitis virus is most stable.

The virus used was the Webster No. 3 strain of St. Louis encephalitis virus.† The suspension of virus used in the study was prepared as follows: Swiss mice (3-4 weeks old) were injected intracerebrally with 0.03 cc of a 10% saline suspension of mouse-brain virus. On the 3rd day following inoculation the mice showed signs characteristic for mice infected with S.L.E. The brains of 5 mice were removed and ground in a mortar with sand and sufficient saline to make a 10% suspension of infected mouse brains. Following centrifugation at 1,500 r.p.m. for 5 minutes, 10-fold serial dilutions of the supernatant fluid were made in saline containing 10% rabbit serum. The virus suspension was then titrated intracerebrally in

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† Kindly supplied by Dr. A. B. Sabin.

TABLE I.
Effect of Hydrogen Ion Concentration on St. Louis Encephalitis Virus.

One cc portions of a 10% saline suspension of virus (intracerebral LD_{50} titer of $10^{-7.8}$) were added to 9 cc portions of a buffer at pH indicated below. Mixtures held at 4°C.

pH of buffer	pH of mixture		Material titrated	Dilutions tested*								LD_{50} titer neg. log. of dilution
	At 24 hrs	At 7 days		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
7.1	7.1	7.1	Mixture after 24 hrs	—	5/5†	5/5	5/5	5/5	3/5	1/5	—	7.3
			” ” 7 days	0/5	0/5	0/5	0/5	0/5	0/5	0/5	—	<2
8.2	7.9	7.9	” ” 24 hrs	—	—	—	5/5	5/5	4/5	1/5	0/4	7.5
			” ” 7 days	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	3.5
9.2	8.7	8.4	” ” 24 hrs	—	—	—	5/5	5/5	4/5	0/5	0/5	7.4
			” ” 7 days	6/6	5/5	5/5	1/5	0/5	0/5	0/5	0/5	4.6
10.1	9.5	8.8	” ” 24 hrs	—	5/5	5/5	5/5	5/5	5/5	2/5	0/5	7.8
			” ” 7 days	5/5	5/5	5/5	3/5	0/5	0/5	0/5	0/5	5.2
11.2	10.8	9.7	” ” 24 hrs	3/4	1/5	1/5	0/5	—	—	—	—	2.6
			” ” 7 days	0/5	0/5	—	—	—	—	—	—	<2

* Dilutions were made in saline containing 10% rabbit serum which possessed sufficient buffering capacity to bring all preparations to about pH 7.5.
† The denominator denotes the number of mice tested and the numerator denotes the number of deaths.

3- to 4-week-old Swiss mice. The LD_{50} titer of the virus suspension was $1 \times 10^{-7.8}$.[7]

Buffers of desired hydrogen ion concentration were prepared by adding either 10% sodium hydroxide or 3 N hydrochloric acid to a solution 0.05 M with respect to glycine and to disodium phosphate. The buffers were then autoclaved for 15 minutes at 15 pounds.

Immediately after preparation 1 cc of the 10% saline suspension of virus was added to 9 cc of buffer of the desired pH (Table I) and the mixture was immediately stored at 4°C. The various virus-buffer mixtures were titrated intracerebrally in 3- to 5-week-old mice after 24 hours at 4°C and again after 7 days. The results appear in Table I. It will be seen that the virus is very unstable at or near pH 11 since very little virus was present after 24 hours. After 7 days no virus could be detected in the buffer solution at pH 7.1 nor in the buffer solution which had an original pH of 11.2. From the data presented it would appear that the virus is most stable at pH 8.8. No further titrations of the buffer-virus mixtures were done but 0.03 cc of the mixtures were inoculated intracerebrally into mice after 2 and 3 weeks at 4°C. After 2 weeks virus was still present in the tubes which had pH values, after 7 days at 4°C, of 7.9, 8.4 and 8.8 but was not detected in the tubes having a greater or lesser hydrogen ion concentration. After 3 weeks at 4°C virus was still present in the tubes which had a pH of 8.4 and 8.8, after 7 days at 4°C, but not in the other tubes.

Summary and Conclusions. From the data presented here it would appear that the hydrogen ion concentration at which St. Louis encephalitis virus is most stable is similar to that reported for the virus of Japanese B encephalitis. The former was found, by titration in mice, to be most stable at a pH value near 8.8. The virus of St. Louis encephalitis could still be detected after 3 weeks at pH 8.4 and pH 8.8 but not at higher or lower pH values.

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