losis. In some instances, such as in renal or cardiac insufficiency, sodium bicarbonate may be contraindicated. It must be emphasized that the purpose of this alkali therapy is to maintain the urine neutral or slightly alkaline, and not to produce alkalosis.

Conclusions. 1. The solubilities of sulfadiazine and particularly of acetyl sulfadiazine, in buffers and in normal urine, increase markedly with increasing pH within the physiological pH range of urine. 2. Examination of urine specimens from patients receiving sulfadiazine has demonstrated that crystalluria due to sulfadiazine can be prevented by maintaining the urine neutral or alkaline and the volume within limits generally considered optimum for patients with infection. 3. This study affords evidence that renal reactions due to the precipitation of sulfadiazine compounds can be prevented by appropriate alkali and fluid therapy. Our clinical findings to date accord with this assumption.

14107

Nutrition of the Golden Hamster.*

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Since several reports have indicated that the Golden or Syrian Hamster (*Cricetus auratus*) may be useful in studies involving human virus diseases,[1,2] we were interested in determining more specifically the nutritional requirements of this animal. It was immediately obvious that the hamster grew very poorly and failed to survive on synthetic diets which allow good growth and reproduction in the rat. The addition of liver extract did not improve the rate of growth appreciably and many animals failed on diets containing several natural products. The animals showed somewhat better growth when the synthetic diet was supplemented with whole liver substance or with preparations from grass.

We wish to report in this paper that one of the limiting factors in purified diets for the hamster is biotin and that when biotin is supplied in adequate amounts together with inositol and para-aminobenzoic acid excellent growth results. Under these conditions neither nicotinic acid nor vitamin C needs to be supplied in the diet.

Experimental. All the animals used were raised in our laboratory from a few stock animals originally obtained from Dr. A. F. Rasmussen, Department of Bacteriology. The stock ration was similar to that used for rats with additional greens and whole corn and wheat. The young were started on experiment at weaning when they weighed 25 to 35 g. The basal synthetic ration was similar to that used in our studies with rats and had the following composition: sucrose 72, casein 18, salts IV 5,[3†] corn oil 2, cod liver oil 2, and wheat germ oil 1. The ration was fed *ad libitum* and 1 drop of haliver oil was given to each animal every 2 weeks. All the vitamins were given in great excess in order to eliminate any possibility that the requirement of

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1 Lennette, E. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 178.

2 Wheeler, A. H., and Nungester, W. J., *Science*, 1942, **96**, 92.

3 Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

† Salt mixture of Phillips and Hart modified by an addition of 5 g $MnSO_4$ per kilo.

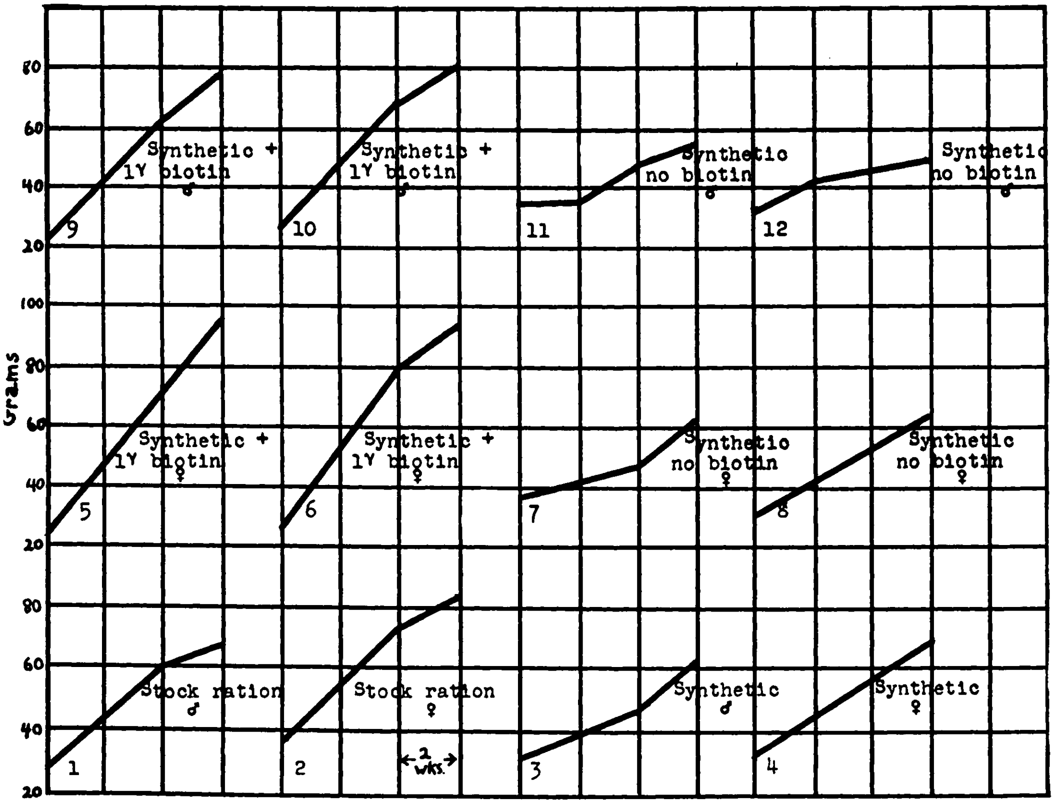

CHART I.
Best pair (3 and 4) on synthetic diet compared with hamsters on stock ration (1 and 2).
Two typical females (5 and 6) receiving biotin compared with 2 typical females without biotin (7 and 8).
Two typical males (9 and 10) receiving biotin compared with 2 typical males without biotin (11 and 12).

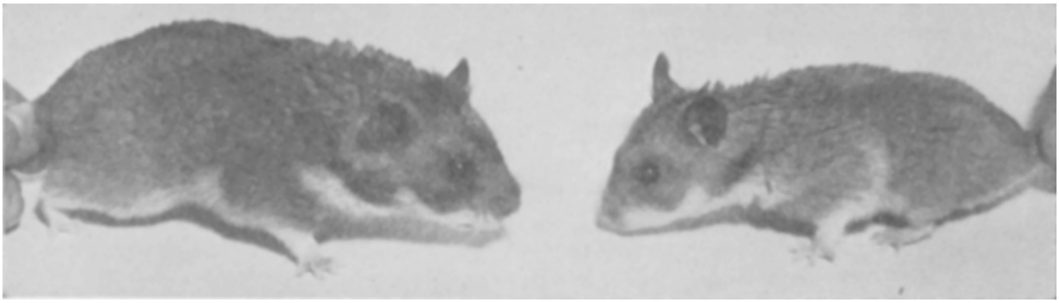

FIG. 1.
Two female litter mates after 7 weeks on experiment. Hamster on left received synthetic diet plus 1 γ biotin daily. Weight 108 g. Hamster on right, synthetic diet with no biotin. Weight 62 g.

the hamster might be higher than for the rat. The daily intake was as follows: thiamine 500 γ, riboflavin 500 γ, calcium pantothenate 500 γ, pyridoxine 500 γ, nicotinic acid 750 γ, sodium para-aminobenzoate 1 mg, inositol 1 mg, and choline 1 mg. The B vitamins were given in 20 cc water in supplement cups daily. In all our early work 1 mg of ascorbic acid was also given each animal daily.

Weanling hamsters placed on the synthetic diet containing the 8 B vitamins and vitamin C grew poorly and many of them died between the 3rd and 5th week after starting on experiment. In one typical series involving 17

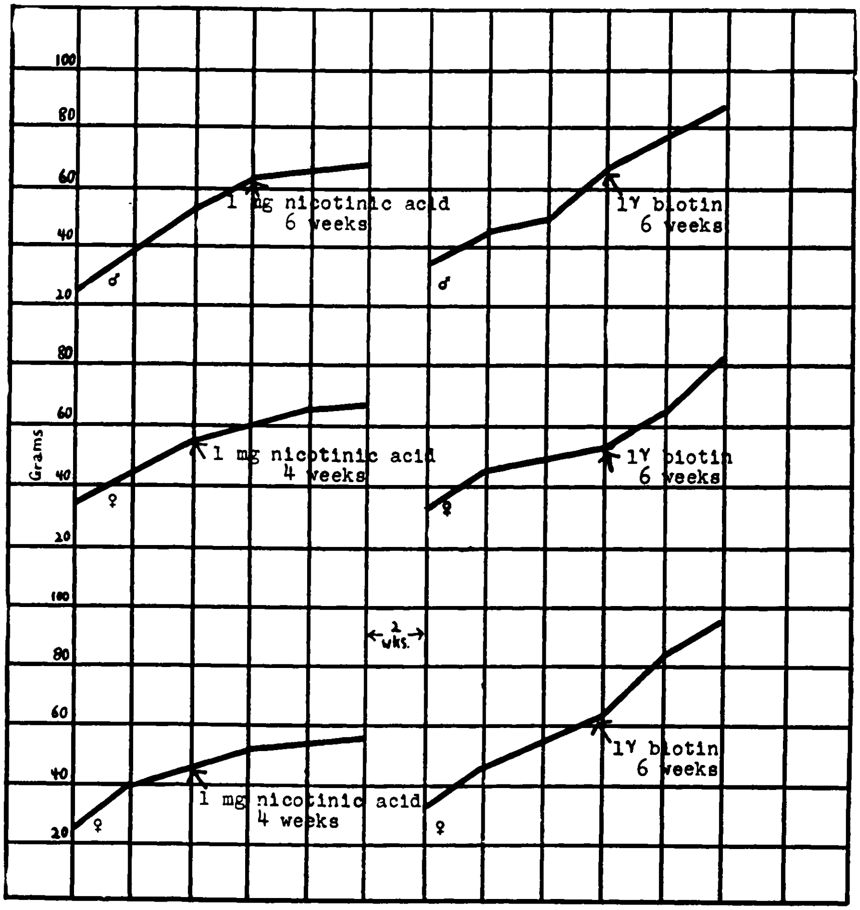

CHART II.
Comparative effects of nicotinic acid and biotin given to hamsters receiving synthetic diet plus ½ γ biotin but no nicotinic acid.

animals, 5 were dead at 4 weeks. The surviving males showed an average daily gain of 0.66 g and the females .95 g during the 4-week period. It is interesting to note that the female hamster grows faster and becomes a larger animal than the male.[4] In Chart I we have compared the growth curves for the best pair of hamsters on the synthetic diet with those for a typical pair on the stock ration. In this case the record approached that of the stock animals but most of the animals on our stock ration have also grown poorly. A few animals from small litters grew fairly well on the synthetic diet for 4 weeks but died suddenly during the fifth and sixth weeks. This higher weight and longer survival time were undoubtedly due to a greater storage of certain vitamins in the animals at weaning. Many of the animals developed a characteristic dermatitis at the corners of the mouth. If the animals lived beyond 6 weeks this dermatitis became fairly extensive.

The addition of 3% liver extract (1-20) prolonged the life of the animals but did not give a significant growth response. Whole liver substance at 3% of the ration produced a growth rate as high as 1.2 g per day for the females. Since liver extract is a good source of "folic acid" and other factors required by the chick these factors could not be the limiting ones for the hamster unless their requirements were exceedingly high. However, whole liver is distinctly higher in biotin than liver extract as shown by microbiological assay and since

[4] Bruce, H. M., and Hindle, E., *Proc. Lond. Zoo. Soc.*, 1934, 361.

TABLE I.
Comparison of Growth Rates on Synthetic Diets and Stock Ration.

	No.	Sex	g per day 4 wk
Basal + 8 vitamins of B group, no biotin	8	♂	0.66
	7	♀	0.95
Basal + 8 vitamins of B group, 1 γ biotin	7	♂	1.12
	13	♀	1.40
Basal + 7 vitamins of B group (no nicotinic acid) + 1 γ biotin	3	♀	1.30
Basal + 7 vitamins of B group (no nicotinic acid), no biotin	5	♂	.80
	2	♀	.92
Stock ration	2	♂	.90
	3	♀	1.00

the animals showed a dermatitis we suspected a possible lack of this vitamin.

As soon as a biotin concentrate‡ was added to the synthetic diet in amounts sufficient to supply 1 γ of biotin per day remarkably good growth was obtained in all cases and no dermatitis was evident. A few typical results are given in Chart I. With added biotin the males showed an average daily gain of over 1 g per day and the females 1.4 g per day during the first 4 weeks on experiment. The animals continued on to maturity and we have carried a few female animals through reproduction with fair success.

When the animals were given the synthetic diet plus 1 γ biotin daily there was apparently no need for nicotinic acid. In a typical experiment 3 females grew very well on a ration deficient in nicotinic acid but complete in all other respects. They gained an average of 1.3 g per day for the first 4-week period and no dermatitis was evident. When both biotin and nicotinic acid were omitted from the daily vitamin supplement the animals weighed less than the animals on a ration containing both these factors. However, controls getting nicotinic acid but no biotin did no better.

Several of the animals were given the synthetic diet with only 0.5 γ biotin and no nicotinic acid. Poor growth resulted so at the end of 6 or 7 weeks half of the animals were also given 1γ biotin, the other half 1 mg of nicotinic acid daily. It is evident from typical cases in Chart II that biotin provided definite stimulation while the nicotinic acid gave little or no effect.

When vitamin C was omitted from the synthetic diet including 1 γ biotin daily growth was no less than when vitamin C was included, the females averaging 1.40 g per day and the males 1.05 g per day for the 4-week period. Vitamin C was then excluded from the daily vitamin supplement of all our experimental animals on the complete synthetic diet and after 6 months there were no indications of any deficiency.

When the animals were given only 6 crystalline members of the B group (thiamine, riboflavin, nicotinic acid, calcium pantothenate, choline and pyridoxine) plus ½ γ biotin daily, three-fourths of the animals died within 3 weeks and the rest did so poorly that they were discontinued from experiment. In another series when the biotin level was raised to 1 γ per day, the animals also did poorly, one-third to one-half dying within 4 weeks. Since animals did very well on rations containing the 6 members of the B group and 1γ biotin plus inositol and para-aminobenzoic acid it is evident that both or one of these factors are necessary for the nutrition of the hamster.

Table I summarizes the comparative rate of growth of the various synthetic diets and the stock ration.

Discussion. Routh and Houchin[5] reported that the hamster needed nicotinic acid for normal growth. Under our conditions we have obtained good growth when nicotinic acid was excluded from the ration of the hamster. Since Routh and Houchin[5] did not include biotin in their rations it seems probable that they were in reality dealing with a biotin deficiency. Mouth lesions which they reported in ribo-

‡ S.M.A. Corp. Conc. No. 200.

[5] Routh, J. J., and Houchin, O. B., *Federation Proc.*, 1942, **1**, 191.

flavin and thiamine deficient animals were encountered by us in biotin deficient animals which had adequate supplies of these 2 members of the vitamin B group so that probably their experiments were complicated by multiple deficiencies.

Our data have also shown that vitamin C can be safely excluded from the hamster's ration since no growth differences were obtained when vitamin C was included or omitted from the diet.

Inositol or para-aminobenzoic acid or both may be needed in the ration of the hamster since deaths occurred when both these factors were excluded from the diet. However, experiments are now in progress to determine whether both or only one is needed.

Conclusions. A synthetic ration containing the 6 crystalline members of the B group which will support growth and reproduction in the rat was found wholly inadequate for the hamster. The addition of para-aminobenzoic acid and inositol increased the survival time of the hamster. A definite need for biotin was shown, the animals growing excellently and reproducing when it was included in the ration along with the 8 members of the B group including inositol and para-aminobenzoic acid. On the complete synthetic diet including biotin, inositol, and para-aminobenzoic acid, vitamin C and nicotinic acid are not required by the hamster.

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Effect of pH on Stability of Vesicular Stomatitis Virus.

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Most viruses are rapidly inactivated at high H ion concentrations, and therefore the report of Galloway and Elford[1] that the vesicular stomatitis virus (VSV) was still active after 24 hr at pH 4.51 and 0°C was of interest. Their results were based on qualitative tests, as a convenient method for titrating virus activity was not available at that time. Recently it has been found here that the 7-day-old chick embryo is quite susceptible to VSV inoculated on the chorioallantoic membrane and that it can be used to titrate the virus. Using the embryo to determine the amount of virus, the pH stability of VSV has been determined quantitatively.

The New Jersey strain of VSV originally obtained through the courtesy of Dr. P. K. Olitsky was employed. Seven-day-old chick embryos were inoculated on the membrane with an egg passage of the virus, and the infected embryos were harvested shortly after death. They were ground with 0.9% NaCl solution to make a 10% suspension by weight and the suspension was centrifuged in an angle centrifuge for 10 minutes at 4000 R.P.M. One cc of the supernatant was then added to 9 cc of buffer of the desired pH. The buffer used was the glycine acetate phosphate buffer described by Northrop.[2] It was chosen because it provides a constant ionic concentration in the medium, except for the H^+ and OH^- throughout the pH range studied. This buffer has been used before for a similar purpose.[3] It was found necessary to use the buffer undiluted, as it did not maintain its pH when the tissue-virus suspension was added if it had been diluted 1 part to 5 with saline (tested colorimetrically).

The buffer samples and the virus suspension were mixed in the cold and kept on ice in the refrigerator. Virus activity was then titrated

* Fellow of The Rockefeller Foundation.

1 Galloway, I. A., and Elford, W. J., *Brit. J. Exp. Path.*, 1935, **16**, 588.

2 Northrop, J. H., and DeKruif, P. H., *J. Gen. Physiol.*, 1922, **4**, 639.

3 Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 49.