results were obtained when young hamsters of uniform size and an age of about 4 to 6 weeks were used.

All 3 of the agents under investigation are known to appear in the circulating blood of certain vertebrates during the course of infection; the equine agents are usually present for only a brief period early in the disease,[9] while the West Nile agent remains for a longer time.[10] The results of experiments designed to determine the distribution of virus in the blood and tissues of hamsters infected with each of the 3 agents are summarized in Table II. It is apparent from the data presented that these 3 viruses appear in the blood of hamsters within 24 or 48 hours after intraperitoneal inoculation; furthermore, that virus is demonstrable in the blood or spleen before it is detectable in appreciable quantities in the brain; and finally, that the late development of large amounts of virus in the brain is associated with a diminution or absence of virus in the blood and spleen.

Conclusions. The Syrian hamster readily becomes infected when small amounts of the viruses of Eastern, Western, or West Nile encephalitis are inoculated by peripheral routes. The viruses appear in the circulating blood of the infected animals and the diseases usually terminate fatally.

The hamster should prove useful in experiments dealing with insect transmission of these 3 agents and with the resistance of treated animals to infection.

14045

Factors Which Interfere with the Benzidine and Meyer's Tests for Blood in Milk and Urine.

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Introduction. A specimen of milk from a cow with mastitis, submitted to the State Hygienic Laboratory for examination, was tinged with blood and contained red corpuscles but was negative to Meyer's test.[1-3] A centrifuged portion was weakly positive and definitely so when the supernatant fluid was replaced by water. The benzidine test[4] gave similar irregularities.

The laboratory also had on record repeated tests on 2 cows which continued to shed red corpuscles in their milk throughout the lactation period. Their milk gave frequent negative tests for blood, becoming positive when the samples were centrifuged and the residues made up to volume with distilled water. Greene[5] has had similar experiences with clinical urine specimens whose microscopic examinations showed large numbers of red corpuscles but which were negative to the Meyer and benzidine tests.

The benzidine reagent may vary in its sensitivity;[6] false negatives may be obtained with small amounts of blood and the test is more sensitive to aqueous solutions than to naturally buffered biological fluids. False positive blood tests may be given by oxyhemoglobin, reduced hemoglobin, methemoglobin, carbon monoxide hemoglobin and acid hematin with both the benzidine and the less

1 Boas, I., *Deutsche Med. Wchnschr.*, 1915, **41**, 549.

2 *Merck Index*, ed. 5, Merck & Co., Inc., Rahway, N.J., 1940, 836.

3 Johannessen, A., *Ugesk. f. Laeger.*, 1921, **83**, 1613.

4 Hawk, P. B., and Bergeim, Olaf, *Practical Physiological Chemistry*, ed. 11, P. Blakiston's Son & Co., Philadelphia, 1937, 397.

5 Personal communication from Dr. J. A. Greene, Department of Internal Medicine, State University Hospital, Iowa City, Iowa.

6 Lyle, W. G., Curtman, L. J., and Marshall, J. T. W., *J. Biol. Chem.*, 1914, **19**, 445.

frequently used guaiac test.[7] False positive reactions may be observed with the benzidine reagent in urine specimens containing large quantities of pus.[8]

Since similar reports regarding milk are lacking, it seemed of interest to study the nature of the factors occasionally inhibiting the reactions and to attempt controlled tests from which the possible inhibitory factors had been eliminated.

Methods. The tests were applied without modification to urine and milk specimens grouped as follows: (1) those found by microscopic examination to be free or almost free of red corpuscles; (2) those known to contain corpuscles, often enough to give color to the sample; and (3) those to which known volumes of washed sheep corpuscles were added, always in amounts exceeding the limiting sensitivity of the test.[2] The sheep corpuscles were prepared according to the method described by Sherwood[9] and were used as suspensions containing 10% by volume of corpuscles. Since the physical status of the cells might be a variable, fragility tests[10] were made and only cells showing normal fragility were added experimentally.

Results and Discussion. The original observations which prompted this study were not exceptional negatives in the presence of visible amounts of hemoglobin. Tests on normal whole milk and urine to which 10% sheep corpuscle suspensions were added did not consistently give positive tests. Corpuscles with fragility values lowered by ageing or by changing the osmotic index of the suspension gave more nearly constant positive responses. Suspensions heated to boiling in a water bath for 5 or 15 minutes also more often gave positive findings than did unheated specimens, provided the samples contained a sufficient concentration of cells, at least 0.5 cc of the 10% suspension in 10 cc of the sample. Although Hastings, Evans and Hart[11] found increases of acidity in milk following enzyme inactivation, the increase was not great enough to influence the test with low red cell concentrations, probably because of the combined buffering action of the milk proteins and salts. Acidity from pH 7 to 5 in either milk or urine increased the occurrence of positive reactions (70% positive), especially if the suspensions were first acidified and then heated after the addition of acid* (90% positive). The acid hematin so produced gave strongly positive tests. The opposite was true if the specimens were made alkaline; the strength of the reaction was reduced or in some cases made negative when the alkalinized suspensions were heated before testing (20% positive).

The removal of casein and fat from milk by the addition of acetic acid also increased the number of positive tests (85% positive). Here also, the corpuscles were lysed by the increased acidity and the freed hemoglobin was available for reaction. Pre-heating before the addition of cells did not influence the final test. Even though some of the corpuscles were removed by entrainment in the precipitation process, sufficient hemoglobin remained in the sample to give a positive test.

Older specimens (12 to 24 hours) gave more positive tests than did fresh specimens. Samples stored at room temperature for the same period gave higher positive yields (60% positive) than those (35% positive) kept at icebox temperatures (4°C). Occasionally, however, entrainment of corpuscles by precipitating phosphates may be partly responsible for negative tests on urine samples known to contain blood.

Summary. 1. When milk and urine specimens containing, in some instances, even macroscopic amounts of red corpuscles, give false negative tests with the standard Meyer or benzidine reagents, the factors found at least partially responsible include: lessened fragility of the corpuscles; increased alka-

[7] Osgood, E. E., *Laboratory Diagnosis,* ed. 2, P. Blakiston's Son & Co., Philadelphia, 1935, 297.

[8] Larson, Kaj., *J. Lab. and Clin. Med.,* 1937, **22**, 935.

[9] Sherwood, N. P., *Immunology,* C. V. Mosby Co., St. Louis, 1935, 374.

[10] Pepper, O. H., Farley, D. L., *Practical Hematological Diagnosis,* W. B. Saunders Co., Philadelphia and London, 1933, 72.

[11] Hastings, E. G., Evans, A. C., and Hart, E. B., *Wis. Agr. Exp. Station Res. Bull.,* 1912, 25.

* 1N hydrochloric acid was used for acidifying all samples.

linity of the specimen, producing alkaline hematin which does not respond; settling or entrainment of red corpuscles by the precipitating phosphates. 2. These factors may be controlled by pre-acidification and heating of the specimen, by storage for 12 to 24 hours at room temperature to increase hemolysis, or by centrifugation and making up to the original volume with distilled water to insure hemolysis. The latter treatment is recommended for urine samples which have been allowed to stand in the refrigerator.

Acknowledgment is due Dr. Ralph H. Heeren of the Department of Hygiene and Preventive Medicine of the State University of Iowa, College of Medicine; Dr. Milford E. Barnes, Director, and Dr. I. H. Borts, Associate Director, the State Hygienic Laboratories, and also to Dr. R. B. Gibson, Associate Professor of Biochemistry, for their valuable aid in this work.

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Attempts to Produce Vitamin Deficiency Diseases by Feeding Compounds Related Structurally to Vitamins.

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It is now well known that it is possible to inhibit bacterial growth by addition to the medium of substances related structurally to certain vitamins. Thus sulfanilamide, which is the sulfur analog of the growth factor *p*-aminobenzoic acid,[1] pyridine-3-sulfonic acid, the analog of nicotinic acid,[2] and N-(β,β-dimethyl-α,γ-dihydroxybutyryl)-taurine, the sulfur analog of pantothenic acid,[3-5] have been shown to inhibit the growth of various bacteria. Since these inhibitions were overcome by supplying additional amounts of the vitamins concerned, it was considered that the inhibitions were due to deficiencies brought about in some way. We have attempted to produce vitamin deficiency diseases of animals by feeding the sulfur analogs of pantothenic acid and of nicotinic acid, and wish to report our observations since they differ from those observed with bacteria.

Symptoms of pantothenic acid deficiency of mice[6] were not observed when the animals were fed the sodium salt of N-(β,β-dimethyl-α,γ-dihydroxybutyryl)-taurine at a level 2000 times greater than the level of pantothenic acid in the ration. (For the sake of brevity, this substance is called sodium thiopanate.) Furthermore, symptoms of pantothenic acid deficiency were no more severe and appeared no sooner in mice fed a ration devoid of pantothenic acid and which contained thiopanate than in those fed the pantothenic acid deficient ration alone. Similarly, pantothenic acid deficiency symptoms were not observed in hamsters fed thiopanate. Both mice and hamsters require pantothenic acid for normal growth and health, and hence it had been expected that the analog which inhibited bacteria that require pantothenic acid would likewise cause disease in animals. However, this did not prove to be the case.

It was not possible to produce a disease in mice by the feeding of large amounts of pyridine-3-sulfonic acid. Since the mouse does not require an external source of nicotinic acid, the failure to cause symptoms of deficiency with this substance may be similar to the failure to inhibit bacteria which do not require the addition of nicotinic acid for growth.[2] With a species like the dog, which requires dietary nicotinic acid, the situation

1 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

2 McIlwain, H., *Brit. J. Exp. Path.*, 1940, **21**, 136.

3 Snell, E. E., *J. Biol. Chem.*, 1941, **139**, 975; **141**, 121.

4 Kuhn, R., Wieland, T., and Möller, E. T., *Ber. deutsch. chem. Ges.*, 1941, **74**, 1605.

5 McIlwain, H., *Biochem. J.*, 1942, **36**, 417.

6 Woolley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 565.