described. 2. The relationship of blood to spinal fluid pyruvate is reported. The amount found in the cerebrospinal fluid is usually 70-120% of that found in a blood sample taken simultaneously. 3. Of the 16 cases with elevated blood pyruvate, 13 occurred in cases of known vitamin B_1 deficiency. These latter constituted the only cases in the entire study with definite clinical evidences of vitamin B_1 deficiency. In the other 3, it is suggested that a relative deficiency of vitamin B_1 may have existed. In 51 additional cases, without clinical evidence of vitamin B_1 deficiency, the blood pyruvate was normal in every instance.

11418 P

A New Type of Vitamin K-Deficient Diets.

S. ANSBACHER.
From The Squibb Institute for Medical Research, New Brunswick, N. J.

Heretofore the vitamin K-deficient Diet E of Almquist and Stokstad,[1] Ration K-1 of Ansbacher,[2] Diet 508 of Dam and co-workers,[3] or modifications thereof, have been used by the majority of investigators[1-7] in assays, in which chicks served as test animals. Since putrified fish meal is an excellent source of one of the natural antihemorrhagic vitamins, obstacles are frequently encountered in the employ of fish meal diets even under conditions tending to minimize bacterial action.

Recently we found that the difficulties arising from K-vitamin synthesis are not experienced when diets are used which contain neither fish meal nor yeast, and in which vitamin K had been destroyed by prolonged heat treatment. We are now making vitamin K assays with Ration K-7 outlined in the accompanying table.

---

[1] Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1936, **12**, 329.

[2] Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

[3] Dam, H., Glavind, J., and Karrer, P., *Helv. Chim. Acta.*, 1940, **23**, 224.

[4] Dann, F. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 663.

[5] MacFie, J. M., Bacharach, A. L., and Chance, M. R. A., *Brit. Med. J.*, Dec. 23, 1939, 1220.

[6] Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 194.

[7] Tidrick, R. T., Joyce, F. T., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 853.

RATION K-7.			%
Heated grain mixture*	wheat middlings	25	83
	yellow corn	58	
Casein†			12
Salt mixture[2]			2
Calcium carbonate			1
Cod liver oil (medicinal)			2

*A mixture containing 25 parts of wheat middlings and 58 parts of yellow corn heated to 120°C for one week.

†To 1.2 kg of G.B.I. Vitamin-free Casein or of casein purified by washing with sodium chloride according to the method of either Supplee *et al.*[11] or Edgar and collaborators[12], an aqueous solution is added containing 20 mg of thiamin chloride, 20 mg of riboflavin, 20 mg of vitamin B_6 hydrochloride and 1 g of nicotinic acid. The product is thoroughly mixed and then dried at a temperature not exceeding 70°C.

It is similar to the Ansbacher *et al.*[8] modification of the 240-H ration of Kline *et al.*,[9] the outstanding difference being the heat treatment of the grain mixture which is prolonged from 36 to about 168 hours.

When baby chicks are fed the heat-treated diet, they grow only very slightly, have an increased blood clotting time and show the typical K-avitaminosis symptoms within from 4 to 8 days, and die within approximately 2 weeks. If it is intended to make vitamin K assays with chicks weighing about 70 g, it is necessary to start baby chicks on a vitamin K-low diet, *e.g.*, Ration K-2,[2] and to feed Ration K-7 or a similar heat-treated diet after the birds attained the desired weight. Vitamin K-deficiency will occur after the fourth day on the K-7 diet, even when the chicks have access to feces, are housed in dirty cages and receive water contaminated with the diet.

Experiments with chicks have shown that Ration K-7 is not only deficient in vitamin K, but also in pantothenic acid and some other as yet unknown factor or group of factors. Therefore, it may be supplemented with a suitable vitamin B complex concentrate, such as *e.g.* an aqueous extract of rice bran similar to that used by Lepkovsky[10] and known to be rich in pantothenic acid.

Preliminary results brought out the fact that Ration K-7 may be used as the basal diet for rats in studies of the anti-achromotrichia factor. When rats were fed this diet from weaning age on, graying of the hair occurred within 60 to 70 days. The absence of the anti-

[8] Ansbacher, S., Supplee, G. C., and Bender, R. C., *J. Nutrition*, 1936, **11**, 529.

[9] Kline, O. L., Keenan, J. A., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1932-33, **99**, 295.

[10] Lepkovsky, S., *Science*, 1938, **87**, 169; *J. Biol. Chem.*, 1938, **124**, 125.

[11] Supplee, G. C., Flanigan, G. E., Hanford, Z. M., and Ansbacher, S., *J. Biol. Chem.*, 1936, **113**, 787.

[12] Edgar, C. E., El Sadr, M. M., and Macrae, T. F., *Biochem. J.*, 1938, **32**, 2200.

achromotrichia factor could not be demonstrated, when the same ration without added crystalline vitamin B_6 was used.

It is to be expected that the new vitamin K-deficient diet will be found a useful tool in studies of some of the biological problems involved in vitamin K metabolism, since the diet apparently does not permit a bacterial K-vitamin synthesis.

11419

A Method for Staining of Carious Lesions in Teeth.*

G. GOMORI. (Introduced by R. G. Bloch.)

From the Department of Medicine, University of Chicago.

In the study of experimental rat caries large numbers of animals must be employed to obtain significant statistical results. As a result the methods for examination of the molar teeth become very important. Some investigators have employed the very tedious method of preparing stained thin-sections by the ordinary technics. Others have attempted to reduce the time and expense by resorting to either gross inspection of the carious teeth or to rapid grinding and examination of ground sections. At the suggestion of Dr. B. F. Miller of the University of Chicago the author has developed a rapid, simple and precise method for the staining of carious areas in rat molars (and also in human teeth). Previously,[1] the author had developed a method for the demonstration of insoluble calcium salts in the tissues. It was found that this method cannot be applied to the study of teeth because the silver solution used in the technic will not penetrate the dense dental tissues. Dentin will get a very superficial black coating but enamel is entirely unstained. However, the surprising observation was made that carious areas stained deep black. This can be explained by the greater permeability to the silver solution of the rarefied carious tissue. That the action of acid actually increases the permeability of enamel and dentin was proved by the following experiment: into healthy, extracted human teeth symmetrical holes were drilled, two into each. One of these

* This work has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

1 Gomori, G., *Am. J. Path.*, 1933, **9**, 253.