TABLE II.
Results of Hemagglutination Tests with Histoplasmin (Lot H-40).

Sera of rabbits immunized with:	Serum dilution										
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
H. capsulatum, G-5	+	+	+	+	+	+	+	+	±	±	—
" " G-6	+	+	+	+	+	+	±	—	—	—	—
C. albicans, M-1*	±	±	±	—	—	—	—	—	—		
S. schenckii, S-7†	±	±	±	—	—	—	—	—	—		
" " S-8†	±	±	±	—	—	—	—	—	—		
6 normal rabbits	—	—	—	—	—	—	—	—	—		

* Agglutination titer with *C. albicans* cells 1:2560.
† Agglutination titer with *S. schenckii* cells 1:2560.

sheep's red cells and therefore could not be used.

As shown in Table II the sera from 2 rabbits immunized with *H. capsulatum* gave titers of 1:1280 and 1:320, respectively. Sera from normal rabbits gave negative results. Sera from 2 rabbits infected with *Sporotrichum Schenckii* and from one rabbit immunized with *Candida albicans* yielded no positive reactions. These sera were shown, however, to contain antibodies against their homologous organisms by standard agglutination tests. Serum from one human case of histoplasmosis showed no antibodies.§

It would seem, therefore, from these preliminary results that this method might be useful in serologic tests for histoplasmosis.

Summary. An hemagglutination test is described using sheep's erythrocytes sensitized with histoplasmin and sera from rabbits immunized with *H. capsulatum*. Sera from normal rabbits gave negative results and no cross-reactions were found with sera containing antibodies against *S. Schenckii* and *C. albicans*.

§ Repeated complement fixation tests of this serum, done by Miss Charlotte C. Campbell, have been similarly negative.

16879

Histological Manifestations of a Magnesium Deficiency in the Rat and Rabbit.*

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According to Tufts and Greenberg[1] two phases are observed in magnesium deficiency in rats. The first is marked by vasodilatation, hyperemia, and hyperexcitability. The second phase is characterized by the development of nutritive failure, cachexia, and kidney damage. Greenberg, Lucia and Tufts[2] showed that prolonged deprivation of magnesium eventually produces degeneration in the kidneys, histologically manifested by degenerative changes in the tubules and progressive calcification in the cortico-medullary zone, and later, in the cortex. Renal damage was also observed in calves maintained on a diet

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1 Tufts, E. V., and Greenberg, D. M., *J. Biol. Chem.*, 1938, **122**, 693.

2 Greenberg, D. M., Lucia, S. P., and Tufts, E. V., *Am. J. Physiol.*, 1938, **121**, 424.

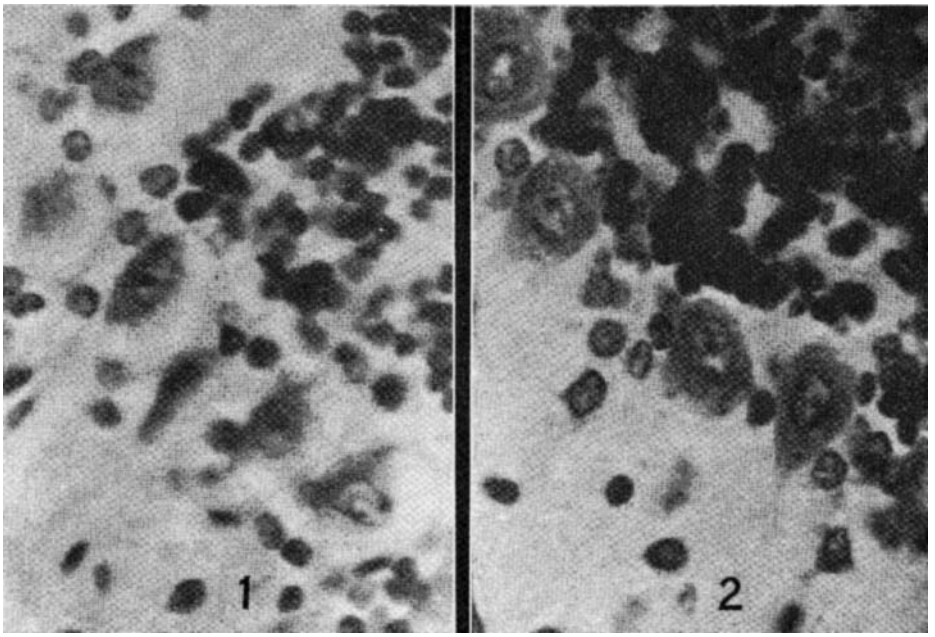

FIG. 1. Cells of Purkinje showing chromatolysis, swelling and displacement of the nucleus in a magnesium-deficient rat.

FIG. 2. Cells of Purkinje from a normal rat. (Einarson's gallocyanin chrome alum stain used in 1 and 2, × 680.)

deficient in magnesium.[3] The most consistent observation was marked proliferation of fibroblasts and fibrosis of the interstitial tissue with marked atrophy and necrosis of the parenchyma. Pathological changes in the heart due to a magnesium deficiency were reported in rats[4] and calves.[3] As Greenberg[5] has pointed out, a lack of certain B-complex vitamins has complicated the interpretations of the manifestations of a magnesium deficiency.

Bird[6] reported cerebellar lesions occurring in young chicks on a diet deficient in magnesium which were observable grossly in the severely affected chicks and only microscopically in mild cases. As far as known, no such lesions have been reported in mammals.

The work reported in this paper was designed to investigate the histological manifestations of magnesium deprivation in the cerebellum of rats and rabbits and in the kidneys and hearts of rabbits.

Experimental. Material. Cerebellar tissue was obtained from young rats that had been restricted to diets containing levels of 1 to 30 mg of magnesium per 100 g of diet for a period of 12 days. Also, cerebellar tissue was obtained from sexually mature rats that had been maintained on magnesium levels of 1 and 30 mg of magnesium per 100 g of diet for periods of 7 and 13 weeks. The dietary regimens and management of the animals has been previously reported.[7,9] In addition, cerebellar, heart, and kidney tissues were obtained from rabbits that had been started on a purified diet at weaning age and maintained on the diet which contained levels of 5 to 85 mg of magnesium per 100 g of diet for a period of 10 weeks.[8]

The brain tissue was fixed in Bouin's solution, absolute alcohol, and a solution of abso-

[3] Moore, L. A., Hallman, E. T., and Sholl, L. B., *Arch. Path.*, 1938, **26**, 820.

[4] Greenberg, D. M., Anderson, C. E., and Tufts. E. V., *J. Biol. Chem.*, 1936, **114**, xliii.

[5] Greenberg, D. M., *Ann. Rev. Biochem.*, 1939, **8**, 269.

[6] Bird, H. F., *Poultry Sci.*, 1946, **25**, 396.

[7] Kunkel, H. O., and Pearson, P. B., *Arch. Biochem.*, 1948, **18**, 461.

[8] Kunkel, H. O., and Pearson, P. B., *J. Nutrition*, 1948, **36**, 657.

[9] Einarson, L., *Am. J. Path.*, 1932, **8**, 295.

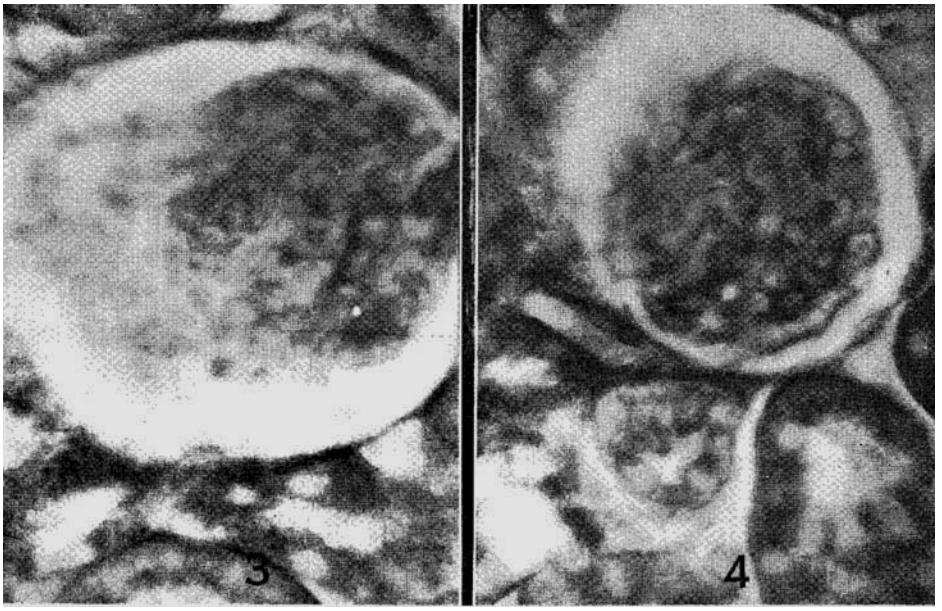

FIG. 3. Swelling and deposition of amorphous substance in the renal corpuscle of a magnesium-deficient rabbit.

FIG. 4. Normal renal corpuscle of rabbit receiving adequate level of magnesium in the diet. (Masson's trichrome stain 1 used in 3 and 4, × 680.)

lute methyl alcohol and 5% glacial acetic acid. Kidney and heart tissue were fixed in Bouin's solution and absolute alcohol. The material was imbedded in a mixture of paraffin and tissue-mat and sectioned at 7 microns. Various staining technics were used, employing silver technics, toluidine blue and erythrosin, hematoxylin and eosin, and Einarson's chrome alum gallocyanin technic.[9]

Histological observations. Histological examination of the cerebellar tissue revealed neuropathological alterations in young rats restricted to diets containing 1 mg and 5 mg of magnesium per 100 g of diet, and also in sexually mature rats restricted to a diet containing 1 mg of magnesium per 100 g of diet. At higher levels of magnesium intake, no cerebellar changes were observed.

The neuropathological changes in the rat were characterized by a degeneration of the cells of Purkinje of the cerebellum. Cytological examination revealed cells in varying degrees of chromatolysis. These cells are characterized, first, by a slight swelling, an increase in volume and a decrease in the staining property of the Nissl substance. In the second stage, there was a peripheral migration of the nucleus and an increase in vacuolation and the cell volume. The nucleus appeared to become more dense with an increase in the affinity for the stain. In some sections a number of shrunken chromatophilic cells could be observed which appeared to be the advanced stage in the degeneration of the cells of Purkinje. These observations on the neuropathological changes afford an explanation of the findings of Greenberg and Tufts[10] that protection against convulsive attacks in the magnesium-deficient rat is not afforded by the injection of magnesium salts. Also, it provides further evidence that the hyperirritability characteristic of a magnesium deficiency is not merely the result of an ion imbalance in body fluids.

Cerebellar sections from rabbits that had been restricted to diets containing 5 and 10 mg of magnesium per 100 g of diet for 10 weeks showed alteration of the cells of Purkinje. These changes were characterized by the presence of a large number of fusiform, dense and basophilic staining cells of Purkinje

[10] Greenberg, D. M., and Tufts, E. V., *Am. J. Physiol.*, 1938, **121**, 416.

without a visible nucleus. Cells with the nucleus displaced to the periphery and varying degrees of chromatolysis were frequent. The swelling that was characteristic of the degenerative changes in the rat was seldom noted. The changes in the rabbit probably represent a more advanced stage of degeneration than the picture presented by the cerebellum of the magnesium deficient rat.

Renal damage could be ascertained in sections from kidneys of rabbits maintained on a diet containing 5 mg of magnesium per 100 g of diet. This damage which involved both the renal corpuscle and tubules was not uniform throughout the kidney but was limited to certain areas of the tissue. Changes were observed in both the cortex and medulla. These degenerative changes consisted of a degeneration of the tubular epithelium and fibrosis of the corticomedullary region. Also in the renal corpuscles the capsule of Bowman remained intact as far as could be observed while the glomeruli were often displaced to the periphery by an amorphous acidophilic staining mass of material (Figures 3 and 4). Such renal corpuscles were often enlarged to as much as twice the diameter of those of the control.

X-ray examination. Sections of 2-4 mμ in thickness were removed and fixed in absolute alcohol. These sections were examined by means of an x-ray machine and plates commonly used in dental examination. Such procedure should reveal the presence of zonary calcification. No zonary calcification was observed by this technic.

Summary. Low levels of magnesium in the diet of rabbits and rats produced chromatolysis and degeneration of the cells of Purkinje of the cerebellum in the rat and rabbit. Nephrosis and fibrosis of the kidney occurs in rabbits restricted to a diet deficient in magnesium.

16880 P

Blood Oxygenation. I. The Kolff Apparatus. II. Multiple Horizontal Rotating Cylinders.*

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I. *The Kolff Apparatus.* In the course of construction of a perfusion apparatus capable of maintaining the circulation of an entire animal, an attempt has been made to use a method of oxygenation without an open blood-oxygen interface.

Experimental. Cellulose sausage casing was wound spirally around a horizontal revolving drum 50 cm in diameter and 50 cm long in a fashion similar to that of Kolff.[1] Casing of the following sizes was utilized: diam. 1.84 cm with 0.203 mm wall thickness, diam. 2.40 cm with 0.203 mm wall thickness, and diam. 4.16 cm with 0.406 mm wall thickness. The diffusion area was approximately 10,000 cm^2. An atmosphere of 95% O_2 and 5% CO_2 was maintained around the casing. Whipped beef blood at 37°C was introduced from a reservoir at one end of the spiral casing and collected at the other end. While the blood flowed through the casing, the drum was revolved at speeds varying between 17 and 100 r.p.m. Blood samples before and after passage through the apparatus were analyzed for oxygen content by the method of Van Slyke.[2] In some experiments the casing dipped into Ringer-Locke solutions to keep it wet. In order to utilize the mixing qualities as well as the diffusion surfaces of the spiral casing, small bubbles of oxygen were in-

* Aided by a grant from the United States Public Health Service, and by a research grant from the Graduate School, University of Minnesota.

1 Kolff, W. J., *The Artificial Kidney*, J. H. Kok, N.V. Hampen (Holland) 1946.

2 Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.