TABLE I.

Group	Supplement	No. of animals used	No. of animals infected with *Bartonella M.*			No. of animals protected against infection
			Died	Recovered	Total	
A	Normal adult rats fed on Purina Dog Chow	20	6	13	19	1
B	Normal young rats fed on Purina Dog Chow	20	18	1	19	1
C	Intraperitoneal administration of Copper in form of CuS (Kupferdiasporal)	10	3	4	7	3
D	Intraperitoneal administration of Iron in form of colloidal highly dispersed ferrous hydroxide (Eisendiasporal)	10	7	3	10	0
E	Intraperitoneal administration of colloidal-highly dispersed Iron-Copper solution	10	5	5	10	0
F	Administration of Ultraviolet Rays	10	8	1	9	1

fected with Bartonella, which was more fatal in the young than it was in the adult animals. In group C, receiving copper alone only 3 out of 10 rats were protected from the anemia. These results resemble somewhat those of Perla, who, however, observed a much better protective effect with copper. Intraperitoneal injection of iron, iron and copper, and iron plus ultraviolet rays, to groups D, E, and F, respectively, did not afford protection to any of the animals.

We conclude from these observations that iron and copper, in doses which prevent and cure nutritional anemia in the albino rat, do not protect the animal against the anemia of Bartonella infection following splenectomy.

7257 C

Pathological Changes in Liver and Spleen in Nutritional Anemia in Rat.

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Pure milk fed to young rats produces severe anemia which finally results in death in the majority of cases. In our studies of the

pathological changes which take place during the course of nutritional anemia in the albino rat, the findings in the liver and spleen seem to have considerable value in the differential diagnosis of this type of anemia as contrasted with the infectious type caused by *Bartonella muris*. All our examined animals showed a severe degree of anemia with an erythrocyte count of less than 3 million per c.mm. and a Hb content of less than 4 gm. per 100 cc. Most of the animals died spontaneously, while 5 were killed during the course of the disease. The spontaneous death of the anemic animals was consistently preceded by a rapid loss of weight during the last days of life. The liver and spleen were weighed and fixed with the other organs in Zenker, alcohol and formalin. Slides for microscopic study were stained with hematoxylin-eosin, van Gieson, Mallory, Bielschovsky, Sudan III and Turnbull's Iron stain.

Changes in the Liver. Macroscopically, the changes were not marked. The size and weight were slightly decreased, the surface smooth and pale, the consistency soft. The cut surface showed a slight yellowish brown mottling and occasional small petechial hemorrhages.

The microscopic changes were most pronounced in those animals which died spontaneously from anemia. The central veins and the capillaries in the center of the lobule were greatly distended and filled with an edematous fluid. In the center of the liver lobule the cells were atrophied; those near the center were swollen and showed a marked vacuolization of the plasma. The majority of these vacuoles proved to be fat droplets when stained with Sudan III. They completely filled the cells surrounding the atrophic areas in the center of the lobule and were present even in livers in which atrophy of the central part of the lobules was just in the beginning stage. The periportal zones showed marked hypertrophy of the cells, with no fat, and an increase in the size of the cell and of the nucleus. Most of the cells contained 2 or more nuclei. Van Gieson, Mallory and Bielschovsky stains showed an early increase of gitter fibres in the center of the lobules which also was present before the atrophy of the center became manifested. The Kupffer star cells and the other endothelial cells were small, the nucleus being pyknotic in many cases. Iron pigment was absent. The periportal collections of small round cells which are so common in the normal rat and guinea pig were entirely absent.

The picture of nutritional anemia resembles that of chronic passive congestion with secondary atrophy of the liver parenchyma. The fatty changes, however, are much more pronounced than those

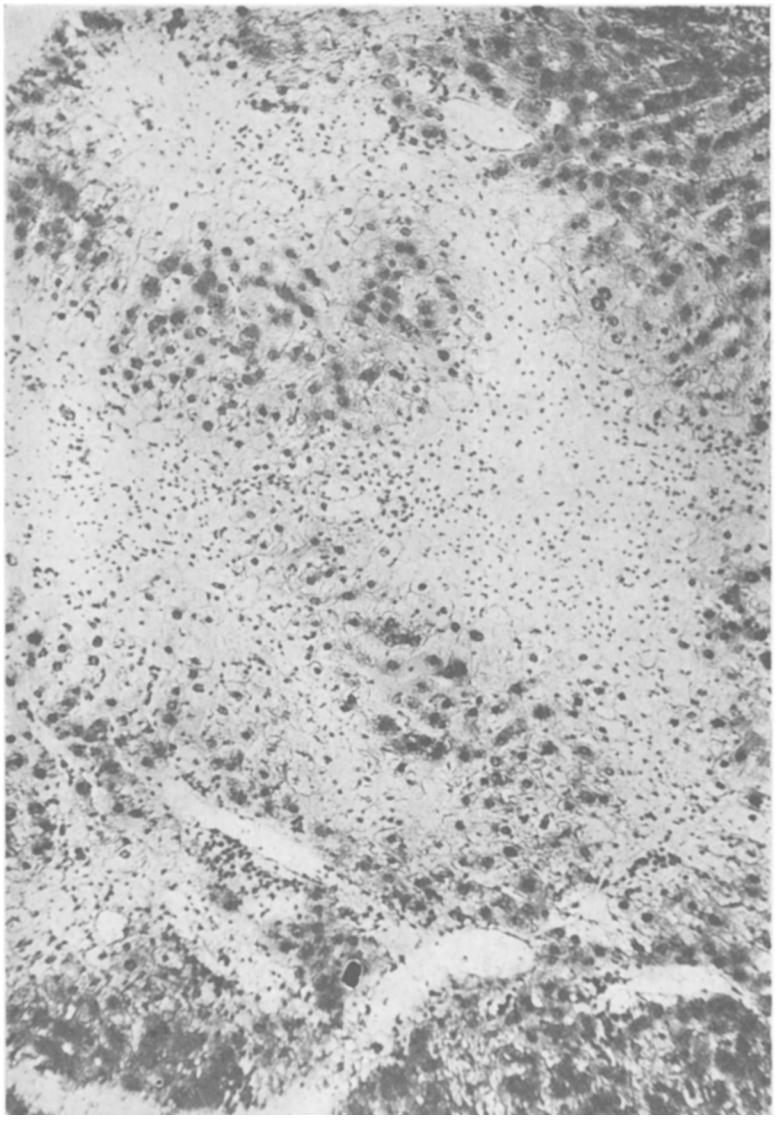

FIG. 1.
Changes in the liver.

found in chronic congestion. The absence of hemosiderin in the endothelial cells and the presence of fluid in the capillaries are more or less typical characteristics of the changes found in the liver during nutritional anemia.

Changes in the Spleen. Macroscopically, no distinct gross path-

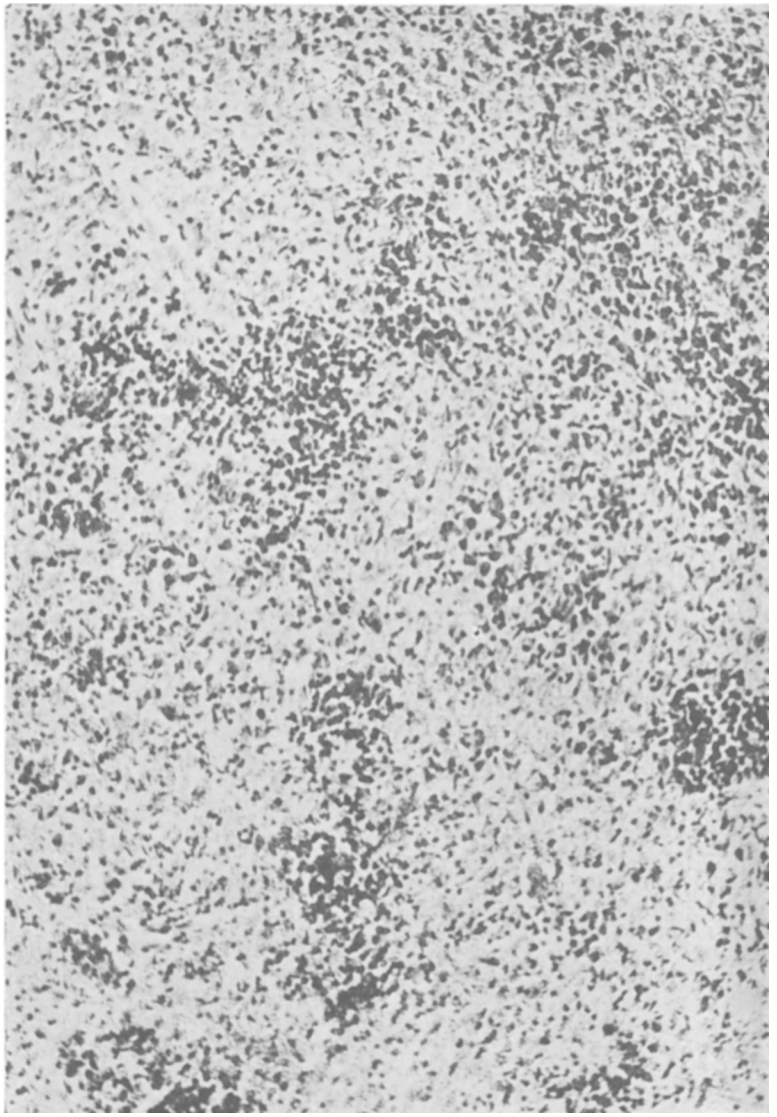

FIG. 2.
Changes in the spleen.

ological changes were present. The spleen was small, pale and soft.

Microscopic examination showed fewer and smaller lymph follicles than those found in the normal organ. There was also a marked decrease in the size of the perifollicular zone of the pulpar cells. The sinuses were dilated and filled with a pink staining fluid.

The pulpar cells showed all the signs of degeneration including complete necrosis with karyorrhexis and pyknosis. The nuclei of the megakaryocytes also showed pyknosis. Iron pigment was absent. In the spleen also, the dissimilarity of the histological picture to that of infectious anemia, with all the signs of splenic hyperactivity, is marked.

Reviewing the changes which take place in the liver and spleen of animals dying from nutritional anemia or killed in the later stage of the disease, we note (1) the absence of any signs of hematopoiesis or hematocataresis, and (2) the presence of severe atrophic disturbances of the cells, associated with signs of failing circulation (chronic heart failure).

7258 P

Use of Living Chick Embryos in the Propagation of B. leprae.

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Because of our failure to obtain *in vitro* multiplication of *B. leprae* in minced chick embryo media,[1] it occurred to us that living chick embryos might afford a more suitable nutritive since the studies of Duval[2] indicate that *B. leprae* is unable to cleave whole protein and that the split products (amino acids) are essential for its multiplication. This hypothesis is substantiated by the observations that the bacilli apparently enter and colonize within the living host cells without destroying them and, as intracellular sojourners, utilize the nutrients intended for the latter; that artificial culture media containing the end products of protein digestion afford a favorable foodstuff for the initial cultivation of *B. leprae;* and that in removed bits of leprous tissue in which autolysis has taken place, the Hansen rods continue to proliferate so long as there are amino-acids present, while in subsequent transplants from these bits of tissue, growth becomes more feeble as the protein split products decrease in amount. Further, the living chick embryo was thought a desirable medium since it is known that there is a large supply of split protein accessible to its growing cells. The question of susceptibility

[1] Holt, R. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 567.

[2] Duval, C. W., *J. Exp. Med.*, 1910, **12**, 649.