retinitis. Toxoplasmata were abundant in the lesions. In this instance, there can be no doubt that the infection was acquired during intrauterine life. Another infant (L.M.) came to autopsy recently with the typical lesions of toxoplasmic encephalomyelitis, which was verified by transmission of the infection to animals from postmortem material. An obstructive hydrocephalus, obviously due to the infection, noted two hours postpartum, points to the prenatal inception of the disease.

In an attempt to ascertain the existence of maternal infection, tests for neutralizing antibodies[14] in the blood of the mothers have been begun. The mothers of the above two infants with proven toxoplasmosis have been studied. Their sera, normal control sera and physiological saline solution were mixed with varying dilutions of mouse brain suspension infected with Toxoplasma, and inoculated intradermally into rabbits. The development of typical Toxoplasma skin lesions was used as an indicator. There was definite inhibition of the formation of such lesions by the sera of the mothers of the infected children. This demonstration of maternal neutralizing antibodies lends further support to the probability that infantile toxoplasmosis is of prenatal orrigin.

This disease of the central nervous system in infants has a specific pathological picture characterized by necrotizing chronic inflammatory lesions, miliary granulomata, conspicuous calcification, frequent hydrocephalus and focal chorioretinitis.[3, 7, 8] The absence of major developmental anomalies of the brain indicates that the infection, when congenital, probably begins late in fetal life.

The symptoms and signs of widespread cerebral damage, roentgenographic evidence of internal hydrocephalus and cerebral calcification, the ophthalmoscopic observation of a striking chorioretinitis, and the presence in the blood of neutralizing antibodies to Toxoplasma, have led to the clinical diagnosis of several new cases also to be reported later. Of these children, two (P.D. and A.I.) have survived to the ages of 4 and 2 years, each giving anamnestic evidence of the disease early in infancy. These children are mentally deficient, and one, subject to convulsions, had been regarded as an epileptic. It is quite probable that such instances of infantile toxoplasmic encephalomyelitis, which survive with severe cerebral damage, may be erroneously classified as congenital malformation of the brain, birth injury, epilepsy, congenital hydrocephalus, etc. It is desirable to separate instances of this specific, congenital infection from these heterogeneous groups, since the hope of successful therapy depends upon their recognition. It would seem especially important to detect the inapparent infection of the mother in order to prevent the disease in the child. It is hoped that serological tests and eventually a curative agent will make this possible.

[14] A. B. Sabin and P. K. Olitsky, SCIENCE, 85: 336, 1937.

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EXPERIMENTAL ANTI-PERNICIOUS ANEMIA FACTOR DEFICIENCY IN DOGS

IN the course of studies on intestinal absorption in dogs with "internal" bile fistulae (anastomosis of the gall bladder to the right renal pelvis with ligation of the common bile duct) we have observed the spontaneous development of an anemia. Further careful study has shown the anemia to be of the macrocytic hyperchromic type.

Normal values for red count, hemoglobin, mean corpuscular volume (MCV), mean corpuscular diameter (MCD) and mean corpuscular hemoglobin (MCHb), have been established, as shown in Table 1. The values for dogs kept 6 months or more on our stock diet do not differ significantly from those of animals newly received.

TABLE 1

ERYTHROCYTE CHARACTERISTICS IN NORMAL DOGS

	Number of observations	Number of dogs	Mean	Standard deviation
Red cell count (millions/mm³)	49	25	6.2	0.7
Hemoglobin (grams per 100 ml.)	49	25	12.9	1.8
Mean corpuscular volume (cubic micra)	49	25	71.3	4.4
Mean corpuscular diameter (micra)	19	18	6.7	0.1
Mean corpuscular hemoglobin (micro-micrograms)	49	25	20.9	1.6

Having established the normal blood picture of dogs in this laboratory it also became necessary to set up criteria for an experimentally produced anemia resembling the human macrocytic hyperchromic anemias, such as pernicious anemia and sprue. It is now generally accepted that this type of anemia in man is due to lack of a factor present in liver, which has been termed the anti-pernicious anemia (APA) factor or erythrocyte maturation factor. Such a deficiency is believed to be produced in man either by failure of intestinal absorption (sprue), lack of intrinsic factor in the gastrointestinal tract (pernicious anemia) or by disease of the liver and consequent inability of that organ to store the APA factor produced by reaction between intrinsic and extrinsic factors (macrocytic anemia of human liver disease). No matter in which way the normal chain of events is interrupted, the result is an increase of MCV, MCD and MCHb above

the normal range, a decrease in the red cell count and megaloblastic or erythroblastic hyperplasia of the bone marrow. Human pernicious anemia and sprue respond to the parenteral administration of liver extract, but are not benefited by any substance of known chemical constitution that has been tested. The criteria for an experimental anemia analogous to pernicious anemia and sprue may therefore be given as: (1) Anemia of the macrocytic hyperchromic type; (2) hyperplasia of the erythroblastic tissue of the bone marrow; (3) response to purified liver extract given parenterally; (4) no response to other factors known to be essential in normal nutrition and metabolism.

Fifteen bile fistula dogs have developed anemia with increases in MCV and MCHb. No dog that has survived the operation for 4 months has failed to develop these blood changes. The highest MCV observed is 98.7; 11 of the 15 dogs have shown MCVs above 88.9 (4 S.D. above the mean for normals). The MCV in every dog has remained above 84.5 (3 S.D. above the mean) for considerable periods. Changes in MCHb have not been as striking as those in MCV. They have exceeded 25.7 (3 S.D. above the mean for normal animals) in 8 dogs and have been above 24.1 in the other 7. In 6 animals the MCD has been determined with a filar micrometer during the height of the anemia and has been found to exceed 3 S.D. of the mean for normal animals in each instance, the maximum diameter attained being 7.4 micra.

This anemia usually appears within 2 to 4 months after operation, shows (like pernicious anemia in man) a tendency to spontaneous remissions with reticulocyte showers, and is accompanied (as in sprue) by marked weight loss.

We are indebted to Dr. L. W. Diggs for reports on the condition of the bone marrow, the specimens being obtained by autopsy or biopsy. He informs us that the marrow exhibits a mild erythroblastic hyperplasia, and that the mean diameter of the nucleated red cells decreases following liver therapy.

The response to liver extract (Eli Lilly and Company, purified) given intramuscularly has been studied in 6 animals. In every instance a single injection of 30 units or more has been followed by a reticulocyte increase (2 to 20 per cent.), and a return of MCV and MCHb to within 2 S.D. from the normal mean. Increases of a million or more cells have been produced, but have required frequent injections totaling more than 30 units.

In order to prevent deficiencies that occur when bile is absent from the gastrointestinal tract it has been necessary to inject vitamins A, D, E and K. If these substances are not given parenterally the dogs are likely to succumb when the red cell count falls below 4.0 millions. In addition we have given various animals injections of thiamin, riboflavin, nicotinic acid, pyridoxin, calcium pantothenate and choline without improvement in the blood picture.

Incidental observations are the presence of acid in the gastric secretion, the absence of plasma bilirubin (which in any case is not detectable in the plasma of normal dogs), the presence of normal concentration of plasma protein and a susceptibility to the development of localized infections at the site of subcutaneous injections.

The anemia in the bile fistula dog fulfils the criteria that we have established for an experimentally produced APA factor deficiency analogous to pernicious anemia and sprue. We believe that this deficiency is probably due to a failure of absorption of the APA factor from the intestine. One must consider that the lack of recirculation of bile might interfere with the functions of the liver, and therefore produce a macrocytic anemia that could best be compared to the macrocytic anemia of human liver disease. But this latter condition usually fails to respond to liver therapy and in this respect differs from pernicious anemia, sprue and the anemia of our animals.

It is evident that a readily available experimental macrocytic anemia responsive to liver extract may make available a method of assay for this extract, which must now be standardized on pernicious anemia patients. We hope that continued study of this macrocytic anemia will also help to clarify the etiology of the macrocytic anemias of man.

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DIFFERENTIAL INHIBITION OF PHOTOCHEMICAL AND DARK REACTIONS IN PHOTOSYNTHESIS BY INORGANIC COMPOUNDS

IT has been known for a number of years that certain organic compounds, notably urethanes and cyanides, are capable of inhibiting one or the other of the reactions in photosynthesis. Studies recently completed have shown that various inorganic compounds can likewise inhibit the light and dark reactions separately. Cells of *Chlorella vulgaris* were suspended in test solutions for 20 minutes. They were then rinsed with water and their rates of photosynthesis were determined over a wide range of light intensities by means of Warburg manometers.

The results are shown diagrammatically in Fig. 1, in which the rate of photosynthesis is plotted as a function of light intensity on logarithmic scales. The control or normal curve is used as the basis of com-