very nearly the same absorption maximum in alcohol as that of a known sample of 4M2M. A negative heat-pink test was obtained with urine of rats, rabbits, hamsters, and a dog that received subcutaneous injections of 4M2MCl solution, but the test was positive after eating of food containing 4M2MCl. The inference that the substance responsible for the heat-pink test was formed in the digestive tract was supported by further observations: A faint pink color was produced when the heat-pink test was applied to samples of 4M2M solution in 0.9% NaCl that had been decolorized in the digestive tract of a rat. A sample of 4M2M solution in 0.9% NaCl, kept over night at room temperature, after addition of a few drops of the liquid from the lower small intestine, then decolorized by passing through cotton, gave a strong heat-pink test. The nature of the substance responsible for the heat-pink test is being investigated further.

Summary. 1. A substantial portion, usually less than half, of the 4M2M, 4M2MCl, 2E2E, and 2M2PM administered to rats and mice in the diet, was found by colorimetric measurements, in the feces. 2. Only a trace of dye color was detected in urine of rats that had received 4M2M or 4M2MCl orally or subcutaneously. 3. Heating, or prolonged exposure to air, of samples of urine from animals that received 4M2M, 4M2MCl or 2E2E orally, not subcutaneously, produced a pink color. The trace of material responsible for this color seemed to be formed from the dye in the digestive tract. 4. Very little unchanged dye was found in the tissues of rats and mice that had received 4M2M or 4M2MCl orally. 5. A large part of the 4M2M or 4M2MCl administered orally is evidently converted into other substances.

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Tissue Abnormalities in Newborn Rats from Vitamin B_{12} Deficient Mothers. (21962)

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Reproductive failure in female rats deficient in vit. B_{12} has been reported by Dryden, Hartman and Cary(1), Lepkovsky *et al.* (2), Richardson, Witten and Couch(3). Thirty to 60% of the offspring died within 3 days and those which survived were underweight and unhealthy. Ferguson and Couch (4) observed degeneration in heart, liver, thyroid and kidney in chick embryos from hens deficient in B_{12}. This suggested that similar changes might be found in rat embryos and might account for their early mortality.

Methods. Weanling female rats were placed on B_{12} low diet of O'Dell *et al.*(5) until 4 weeks before mating when they were transferred to experimental diets. Basal diet was synthetic and contained 25% commercial soybean protein* extracted 6 times by boiling

* The Drackett soybean protein C-1, the Drackett Co., Cincinnati.

Tissues from newborn rats born to vit. B_{12} deficient mothers compared to those derived from control litters.

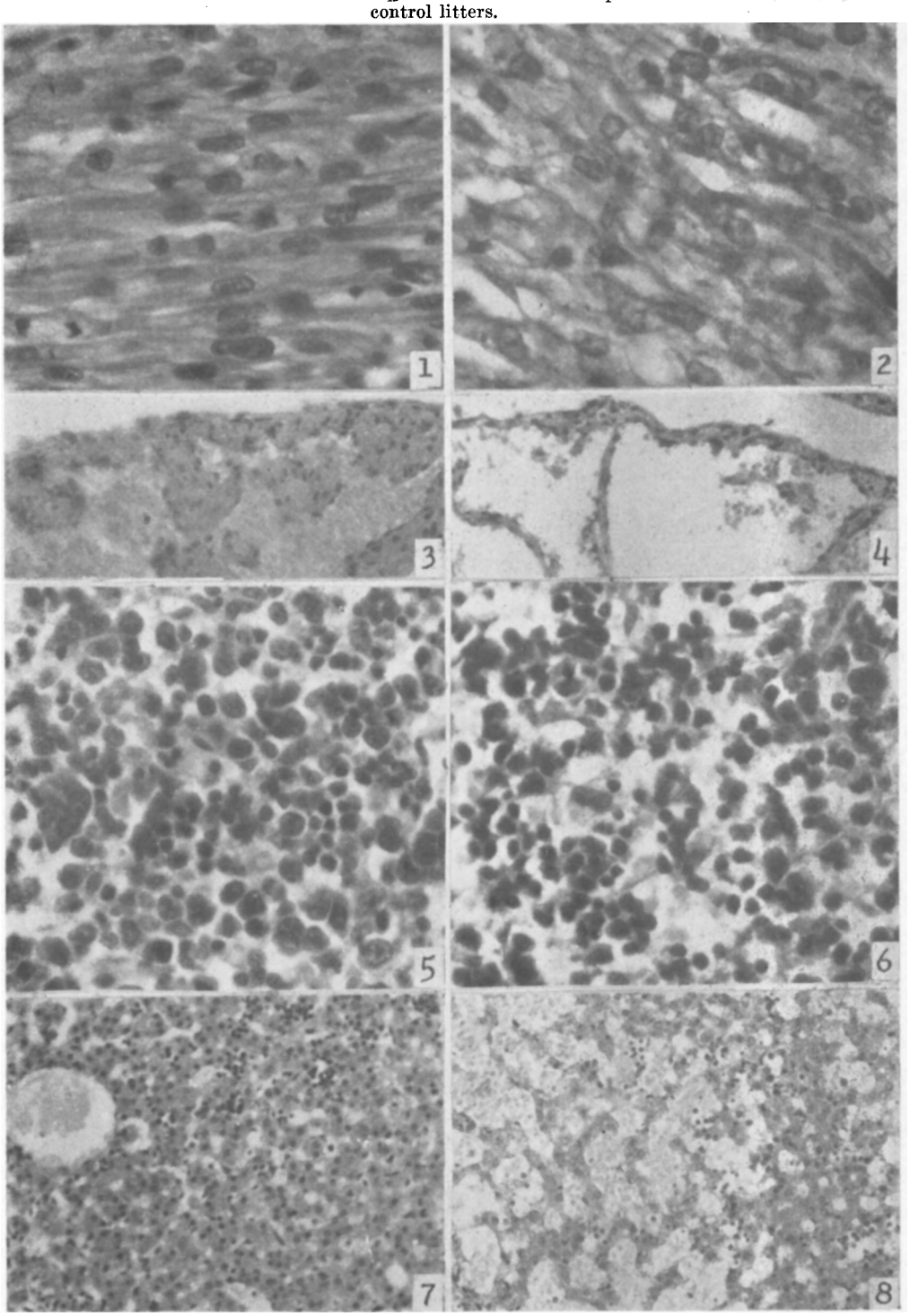

FIG. 1. Longitudinal section of heart muscle from control rat weighing 7.4 g. Chromatic nuclei and basophil cytoplasm. × 720.

FIG. 2. Longitudinal section of heart muscle from experimental rat weighing 2.7 g. Less chromatic nuclei, less differentiated cytoplasm and cell arrangement. × 720.
FIG. 3. Thick wall of auricular appendage from control rat. × 450.
FIG. 4. Very thin auricular wall from experimental rat of 2.7 g. × 450.
FIG. 5. Spleen of control rat with reticular cell background. × 720.
FIG. 6. Spleen of experimental rat (wt 4.15 g) lacking reticular primordial cells and filled with dense pyknotic nuclei with little cytoplasm. Population density of cells is low. × 720.
FIG. 7. Control liver with narrow uniform sinusoids basophil liver cells and numerous blood islands. × 450.
FIG. 8. Liver from experimental rat of 4.7 g showing dilated sinusoids filled with ghost red cells and fat. Note lack of basophilia and diminished size of liver cells. × 450.

70% isopropyl alcohol. One group received basal diet alone. The control group received a supplement of 25 μg of vit. B_{12}/kg basal diet. The vitamin-deficient females weaned only 40-70% of their young while controls weaned 95%. The young within 3 to 4 hours of birth were killed by ether and injected and fixed whole in either Bouin's solution or neutral formalin, or a mixture of 1 part formalin to 9 parts alcohol for study of glycogen. Ninety young from deficient mothers and 22 from control mothers were used. Hearts, livers, kidneys and spleens were excised, weighed and studied histologically in 7 μ paraffin sections. Other portions were sectioned at 13 μ in freezing microtome. Qualitative histochemical staining tests were used as follows: lipoids; Sudan IV on frozen sections; amyloid; dahlia or methyl violet(6); glycogen; Bauer-Feulgen; ribonucleic acid; toluidin blue with enzyme treated controls; desoxyribonucleic acid; methyl green pyronin(7).

Results. Structure and histology of organs of B_{12} deficient young. Experimental newborn rats were underweight, average 4.75 g (2.14-5.18) while control rats averaged 6.0 g (4.3-8.0). The gestation period for both was 21 days. Weight of heart, liver and kidney of experimental rats showed that whereas the former were below normal weight, they were heavier than normal in proportion to the rest of the body. The above are characteristic of immature animals.

Heart. Aortic arches showed no anomalies, although the chambers of the heart were not examined. The structure of muscle cells in both auricle and ventricle showed a fetal pattern, with little basophil cytoplasm, poorly developed myofibrillae and striations, and marked cell borders (Fig. 1, 2). Sudan staining showed small lipoid vacuoles in the cytoplasm. A few lymphocytes were seen in connective tissues. The figures compare longitudinal sections of ventricular muscle photographed under identical conditions. Nuclei were larger but less chromatic with a perinuclear ring of chromatin. D.N.A. determinations showed less nucleoprotein. Auricles were thin, not because of distension but because of failure to develop full thickness of wall (Fig. 3, 4).

Spleen. The control spleen showed a background of reticular cells against which small lymphocytes were contrasted. In the deficient animals the reticular cells had generally disappeared leaving a reticular net of fibers among which were many large, very dense nuclei, often pyknotic. These cells had little cytoplasm. Some cells with more cytoplasm contained hemosiderin (Fig. 5, 6).

Liver. In contrast to the fairly uniform sinusoidal pattern of normal liver, the liver of deficient young under low power showed a dilation of sinusoids between periphery and central veins. The dilated peripheral portions of the sinusoids contained normal non-nucleated red cells. Then followed a zone of red cell ghosts and fragments. The more central portions were plugged with fat globules of undetermined origin. Finally, the zone immediately around the central vein was packed with various leucocytic cells (Fig. 7, 8). This blockage of liver circulation resulted also in shrinkage of liver cords, disappearance of glycogen and nuclei with reduced D.N.A. Von Kupffer cells were all enlarged and contained much blood pigment. Hemopoietic islands of the liver were still present, but the cells, like those of the spleen, had large, dense or even pyknotic nuclei with little cytoplasm. (Fig. 9, 10, 11).

Kidney. The kidney of experimental animals showed retardation of growth more than any other organ. The cortex was of fetal

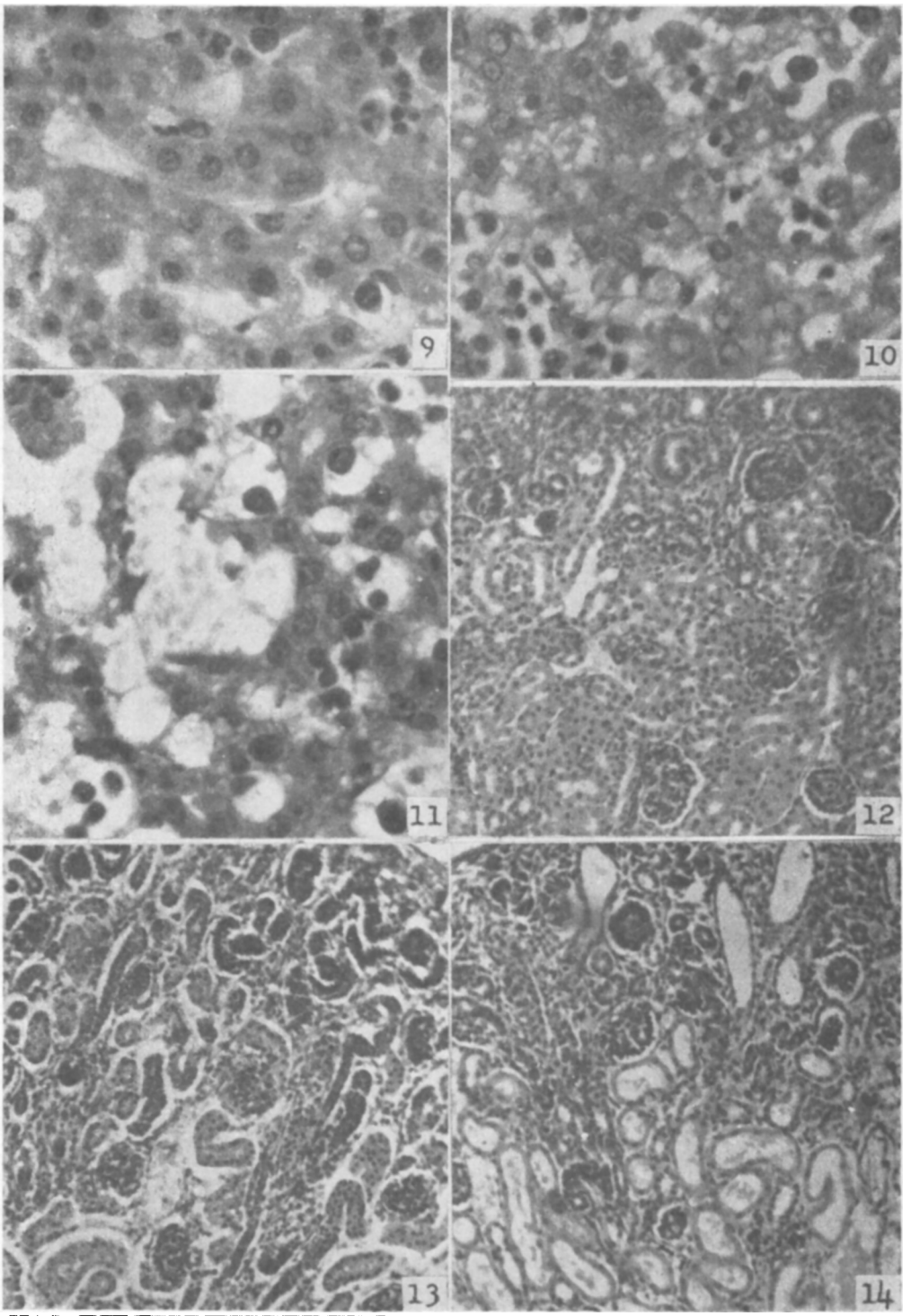

FIG. 9. Control specimen of liver with variable but chromatic nuclei and abundant cytoplasm. × 720.

FIG. 10. Liver from experimental animal of 2.7 g with loss of chromaticity in liver cell nu-

clei, loss of cytoplasmic volume and basophilia and transformation of both blood islands and cells of von Kupffer into pyknotic cells. × 720.

FIG. 11. Liver from deficient rat of 4.2 g wt with great dilation of sinusoids filled with fat and fragmented red cells and fading out of many liver cell nuclei. × 720.

FIG. 12. Cortex from kidney of a control rat (wt 7.4 g) showing one row of undifferentiated glomeruli and tubules. × 450.

FIG. 13. Kidney of deficient rat of 2.7 g showing several laminae of undifferentiated glomeruli and tubules and approximately 2 functional rows deeply placed. Lumens are not clearly defined. × 450.

FIG. 14. Kidney cortex from a deficient rat of 4.05 g showing great dilation of differentiated tubules and reduction of brush border or failure to develop such a border. × 450.

structure filled with incompletely formed glomeruli and tubules. The smallest animals showed the most immature picture (Fig. 12, 13, 14). In addition to the fetal picture, the convoluted tubules of young rats weighing about 4 g showed great dilation of tubules (Fig. 14), the capillary ducts and pelvis of the kidney. The brush border had either sloughed or had never formed. The lumina contained no detritus. Bowman's capsules also were dilated but were still functional. The tubules all resembled distal convoluted tubules. Sudan staining showed fine basal lipoid vacuoles in the tubular cells not seen in controls. On the other hand glycogen was lacking. Vascular extravasation was present together with extratubular edema. Newborn young weighing about 2.7 g showed a much less developed kidney in which no lumen was present in the tubules except in deeper, more mature and functioning units (Fig. 13). Here also was much extratubular fluid. In all of these abnormal kidneys there was abundant medullary mesenchyme as in the fetus but no amyloid deposits.

Discussion. It is a general fact that female rats placed on a low B_{12} diet show little evidence of abnormality in their tissues(8,9) but do develop deficiencies in their reproductive processes and severe growth deficiency in their young(1,3). The degree of growth deficiency and of pathology were coordinate with the immature birthweights, the smallest showing most severe effects. The work of many authors dealing with vit. A deficiency(10,11) with blocking agents like urethane, galactoflavin(12), methylene blue, irradiation(13), etc., show that whatever specificity these agents seem to have lies in the particular moment of susceptibility of tissues at the time of impact of the reagent or deprivation of some essential nutrient.

The case of B_{12} deficiency in addition to a possible correlation above showed that some of the results seen after birth were the indirect result of blocks in liver or kidneys. Similarly, cardiac anomalies were held responsible for urogenital hypoplasias in the work of Baird *et al.*(12) using galactoflavin.

There may be additional more specific effects in the present experiments because of the probable B_{12} requirement for synthesis of nucleoprotein(8,9). Hematological work on bone marrow and spleen is needed. Our sections showed great variability in cytoplasmic basophilia in erythrocytes. There was no anemia or general hypoxia in the mothers to indirectly affect the young.

Liver damage has been reported in swine (14) and in adult rat(9) as a result of feeding a B_{12} deficient diet. Ferguson and Couch(4) reported an increase of fat content in cells of heart muscle, in kidney tubules and in hepatic cells of chick embryos from hens on a diet low in vit. B_{12}. In addition, they reported damage to liver cells beyond this. These cell changes could be prevented by injection of B_{12} into the egg or into the deficient hens some time before the eggs were laid. The above seem to support the view of Ling and Chow(15,16) that this vitamin directly or indirectly plays a part in the accumulation and transformation of glycogen and fat.

The probable role of vit. B_{12} in protein metabolism and particularly in nucleoproteins was best seen in heart muscle. The cytoplasmic deficiency could have been due to overwork but was probably a part of the picture of immaturity. The failure to find any amyloid in connective tissues of the body also points to immaturity.

The abnormal changes in the heart, liver, kidney and spleen noted in our experiments with rats were entirely prevented by supple-

menting the deficient maternal diet with vit. B_{12}.

Conclusions. Female rats deprived of B_{12}, from before the time of mating until the end of gestation, produced deficient and weak progeny. This was shown in weights well below normal and arrested development proportionate to the subnormal weights. The defects involved vascular blocks especially in the liver with consequent passive congestion. The heart and kidneys were immature. Proximal convoluted tubules became blocked and transformed into distended tubules histologically like distal tubules. Spleens showed a loss of primitive reticular cells and transformation into large densely chromatic and pyknotic cells. The origin of the fat by which the liver sinusoids were blocked is not known.

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Accumulation of Homologous Radioiodinated Albumin in Experimental Tumors. (21963)

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Heterologous proteins and amino acids have been shown to localize selectively in transplantable and spontaneous tumors of animals(1,2). This study was undertaken to determine whether homologous proteins are concentrated in tumor tissue since homologous albumin (human serum albumin) tagged with radioactive iodine is used extensively as a diagnostic tracer agent for the detection of brain tumors and metastatic lesions of the liver(3).

Several attempts to measure the distribution of radioactivity in biopsy specimens from tumors in humans have been inconclusive because of low activity in the tissues when permissible tracer doses were used. Accordingly, it was decided to study the problem in animals inoculated with experimental tumors, using homologous albumin.

Method. Rabbit albumin was obtained by ammonium sulfate separation, dialyzed and immediately lyophilized. Electrophoretic patterns indicated the material to be albumin, the single characteristic peak being visible. The albumin was trace iodinated with I^{131} according to the procedure of Gleason and Tabern (4), and yielded a protein of high specific activity (100-600 μc/ml with an albumin content of approximately 10 mg/ml). Ultracentrifugation and electrophoretic analysis have