TABLE II. Identification of Hemoglobin in Irradiated Mouse Chimeras.

Group	No. of recipient mice*	Dose (r)	Donor nucleated cells ($\times 10^6$) Bone marrow† C57BL	Bone marrow† 101	Spleen‡ C57BL	Spleen‡ 101	Post-treatment (mo)	Chimera hemoglobin — Electrophoretic mobility§	Chimera hemoglobin — Optical density‖
A	2	900	15				3	Single	.039 ± .011
	5	500			120		3	"	
B	16	900		15			2–3	Diffuse	.18 ± .008
	1	600				120	3	"	
C	13	400–600			120		2–4	"	.40 ± .012
	10	"				30	2–3	"	
	3	600				12	2	"	

* (C57BL × 101)F_1; 12-16 wk old. † Inj. intrav. ‡ Inj. intraper. § As determined by starch gel electrophoresis, nomenclature of Huecksohn-Waelsch *et al.*(5). ‖ Avg O.D. ± S.E. of mouse HbCO filtrate (see text).

HbCO and for equal mixtures of single with homozygous-diffuse HbCO (Fig. 1) suggests that each hemoglobin gene expresses itself in hybrids by controlling the synthesis of its own hemoglobin type. The development of crystals of single-type hemoglobin in mixtures of HbCO of different genotypes (see Figs. 2, 3, 4) is consistent with this hypothesis. In this respect the action of the hemoglobin genes of the mouse is similar to that of genes governing hemoglobin synthesis in man(6,7).

Summary. Genetically different mouse hemoglobins differ in solubility and crystal formation. Differences in hemoglobin solubility and crystal formation can therefore be used to identify homologous erythrocytes in irradiated mouse chimeras if the hemoglobin types of donor and recipient mice are distinguishable by these properties to begin with.

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Effect of Dietary Zinc Deficiency of Chicken Hepatic Cells. (25085)

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O'Dell and Savage(1,2) reported that growth of chicks was stimulated when as little as 6.6 ppm of zinc was added to a purified diet of glucose-isolated soybean protein (Drackett Assay Protein C-1) type even though the basal contained about 50 ppm of Zn. Further reports concerned with zinc requirements of chicks and poults fed highly refined diets have been reported by Norris *et al.*(3), Morrison and Sarett(4), Pensack *et al.*(5), Norris and Zeigler(6), Roberson and Schaible(7) and Supplee *et al.*(8). This communication reports histopathological findings observed in chicks fed a diet deficient in zinc 14 days following hatching.

Materials and methods. Crossbred male

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TABLE I. Size of Nucleoli of Hepatic Cells as Related to Supplemental Zinc, in 20 Birds.

No supplemented zinc		40 ppm supplemented zinc	
	Nucleoli (μ)		Nucleoli (μ)
R-1	3.80	R-1	2.29
	4.04		2.41
	3.82		2.20
	4.01		2.24
	3.79		2.20
	3.89		2.27
R-2	4.02	R-2	2.23
	3.96		1.97
	3.81		2.03
	4.58		2.35
	3.93		2.43
	4.06		2.20
Avg	3.97	Avg	2.23

chicks from a mating of New Hampshire males to Columbian females were fed experimental diets in metal batteries, coated with plastic resin. A purified basal diet (Drackett Assay Protein C-1-Cerelose) was used. Feed and distilled water in glass containers were offered *ad lib.* for 14 days. The basal diet contained 13 ppm of zinc. It was supplemented with 40 ppm of zinc from zinc carbonate. Five chicks from each of 2 replicates were examined for histopathologic changes. Portions of heart, spleen, liver, kidney and small intestine were collected and fixed in 2% acetated, 10% formalin, Bouin's and Carnoy's solution. Tissues were processed by paraffin method in autotechnicon and stained with Harris' hematoxylin and eosin.

Results. Histopathologic observations. Only liver tissue presented histopathologic changes that could be related to dietary treatments. Enlargement of nucleoli of hepatic cells of zinc deficient birds was apparent upon casual observation of slides and this was confirmed by quantitative measurements. No differences were seen in size of nuclei between treatments.

Hepatic cells of zinc adequate chicks appeared normal while those of zinc deficient chicks appeared to have nucleoli almost twice as large as nucleoli in zinc supplemented chicks. The nucleoplasm of hepatic cells in zinc deficient birds appeared normal in other respects.

Normal and abnormal hepatic cells were observed in all slides but in varying proportions. To measure quantitatively diet induced differences in size of nucleoli of hepatic cells, 100 nucleoli were measured on each coded slide, selected at random. The method used to measure quantitative differences is similar to that employed by Jungherr *et al.*(9). There were 10 slides or a total of 1000 nucleoli measured/lot. A Spencer micrometer and 60X Bausch and Lomb objective were used. Nuclear components were considered circular and the largest diameter at sharp focus representative of size. Quantitative data significant at 0.1% level supported preliminary observations. Nucleoli of zinc adequate chicks had a mean diameter of 2.23 μ and those of zinc deficient chicks 3.97 μ or 78% larger.

Distributions of nucleolar sizes are shown in Table I.

The differences between zinc deficient and supplemented lots were considered pronounced in regard to size of nucleoli of hepatic cells.

Jungherr *et al.*(9) were the first to report nucleolar hypertrophy of hepatic cells of the chicken as a result of a nutritional factor; namely, an arginine deficient diet. They reported enlarged nucleoli in hepatic cells of arginine deficient chicks and observed that the alteration was uncommon in routine avian necropsy material and in Exp. A, D, and E hypovitaminosis of chickens. Nucleolar hypertrophy of hepatic cells was observed in rats fed toxic doses of thioacetamide(10) and in regenerating hepatic cells of rats that had been subjected to partial hepatectomy(11).

Summary. Hepatic cells of chicks fed a purified basal diet consisting of isolated soybean protein (Drackett Assay Protein C-1) with no supplemental zinc, exhibited nucleoli 78% larger than nucleoli of hepatic cells in equal number of chicks fed the same diet supplemented with 40 ppm of zinc. No other histopathologic changes were observed in sections of heart, spleen, kidney and small intestine.

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Reaction of An Aspergillus Polysaccharide with Cryptococcus Capsules and Various Acidic Polysaccharides.* (25086)

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Recently, a polygalactosamine (galactosaminan) has been isolated from cultures of *Aspergillus parasiticus* by electromigration(1,2). This polysaccharide contains only galactosamine, 35% of which is acetylated(2), and when a solution of the polysaccharide is exposed to an electric current in an electrophoresis or electrodialysis apparatus it moves toward the cathode, presumably due to the positive charge conferred by non-acetylated galactosamine. The capsular polysaccharide of *Cryptococcus neoformans* moves to the anode under similar conditions due to its content of glucuronic acid(3,4). That the first of these polysaccharides possesses positively charged amino groups led to the prediction that it might react with the carboxyl groups of the *C. neoformans* polysaccharide, or, for that matter other acidic polysaccharides. Such a reaction might result in precipitation if both polysaccharides were in solution when mixed or in a "capsular reaction"(5-7) if the acidic polysaccharide were in the form of a capsule surrounding a microorganism. Such reactions occurred and a preliminary study of the interaction is presented here.

Materials and methods. Samples of polygalactosamine (APS) from *Aspergillus parasiticus*(2) were furnished by Dr. Saul Roseman, Univ. of Michigan. Stock solutions were prepared by dissolving APS (1 mg/ml) in M/100 HCl or M/20 H_3PO_4. Dilutions of the stock were made in water, saline solution, or buffer. Capsular polysaccharides of pneumococcus types 2 and 3 (S2 and S3) were isolated from broth culture as described in(8). Commercial (USP) grade gum acacia was used without further purification. Samples of dextran were kindly furnished by Dr. Allene Jeanes. Cryptococcus polysaccharides were precipitated with ethanol in presence of acetic acid and sodium acetate(4,9). Samples of Type A polysaccharide (SA) used were further purified by electromigration in an electrodialysis chamber(4). Each of the 3 cells contained 100 ml of veronal buffer pH 8.6 or acetate buffer pH 5.0 (0.025M). In addition, the center cell contained SA dissolved in buffer at a concentration of 1 mg/ml. A current of 100 m.a. was applied for 5 to 6 hours and then the SA was deposited in a gelatinous layer on the anode membrane and was scraped off and dissolved in water for final ethanol precipitation. For precipitin tests, all polysaccharides were dissolved in 0.9% sodium chloride solution. Preliminary tests indicated that good precipitation of acidic polysaccharides could be obtained with as little as 50 μg APS/ml; therefore 0.5 ml APS (100 μg/ml) was added to each 10 x 75 mm test tube followed by 0.5 ml of acidic polysaccharide or dextran at various concentrations. Tests were read after

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