by finding that thyroxin induced a greater erythropoietic response in thyroidectomized rats than did dinitrophenol even when the latter drug induced a higher oxygen consumption, and 4) by noting that thyroxin treated thyroidectomized polycythemic rats responded by an elevation in Fe59 uptake while dinitrophenol in similar rats had no effect.

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Development of Hepatic Phosphorylase in the Chick Embryo.* (29627)

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Experiments performed during the past few years on the relationship between the embryologic development of insulin and glucagon and the synthesis and breakdown of glycogen have provided evidence that, in the rat embryo, glucagon-like phosphorylase-activating substances and phosphorylase activity develop in a parallel fashion reaching maximum concentrations shortly before birth. Approximately at the same time, hepatic glycogen concentration decreases abruptly(1-6) and blood glucose concentration increases(7). Nemeth and collaborators(8) reported that, in the guinea pig, hepatic phosphorylase may be demonstrated several days before the beginning of glycogen deposition. On the other hand, Bot and collaborators(9) and Grillo (10,11) have reported that, in some tissues of the chick embryo, glycogenesis antedates the appearance of demonstrable phosphorylase activity. These results suggest that the phosphorylase-mediated pathway may not be directly related to glycogen synthesis in these embryonic tissues. Ballard and Oliver(12) found measurable phosphorylase activity in the liver of chick embryos as early as the 11th day of incubation, but did not examine younger embryos. Leibson(13) reported that epinephrine injections cause a significant decrease in liver glycogen before day 14, while, according to Thommes and Firling(14), glucagon is effective as early as day 6. Since glycogenolysis induced by epinephrine or by glucagon requires the activation of phosphorylase, these results suggest that this enzyme may be present in the liver of the chick embryo earlier than suggested by previous studies. The purpose of this work was to reinvestigate the problem of the ontogeny of hepatic phosphorylase in the chick embryo.

Materials and methods. Fertile chicken eggs (White Leghorn, Carruthers Farm, Bancroft, Mich.) were incubated at 37.5 ± 0.3°C in a commercial farm incubator. When

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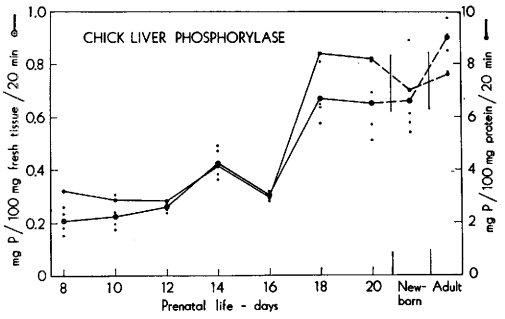

FIG. 1. Phosphorylase activity in chick liver. Lines are drawn through average values. Small points represent individual determinations per 100 mg of tissue.

desired, the eggs were opened, the embryos transferred to a watch glass, the liver removed, blotted, weighed on a torsion balance, and homogenized as described below.

Hepatic phosphorylase was determined by measuring the rate of liberation of inorganic phosphate from glucose-1-phosphate (G-1-P) during the synthesis of glycogen, using a modification of the method of Hers(15). For this purpose, a 10% (w/v) homogenate of liver in ice-chilled 0.05 M Tris buffer containing 0.005 M EDTA, pH 7.8, was prepared by means of a glass homogenizer and centrifuged at 0°C. The supernatant was diluted with 9 volumes of the same cold buffer and 0.05 ml portions of it were pipetted into 4 centrifuge tubes. After adding 0.05 ml of a reagent containing glucose-1-phosphate 0.1 M, 5-AMP 0.003 M, NaF 0.2 M, and glycogen 2%, pH 6.1, the samples were removed from the ice bath and incubated at 37°C. Two samples were incubated for 10 minutes, the other 2 for 20 minutes. Incubation was stopped by adding 0.2 ml of 10% trichloracetic acid, followed by 4.0 ml of distilled water and 0.5 ml of 2.5% ammonium molybdate in 5 N H_2SO_4. The tubes were centrifuged for 10 minutes at 2500 rpm in a refrigerated centrifuge and the supernatant decanted into a colorimeter tube. Two-tenth ml of phosphate reagent (sodium bisulfite 14.6%, sodium sulfite 0.5% and 1,2,4-aminonaphthol sulfonic acid 0.25%) were added and, after 10 minutes, the color was measured at 660 mμ. The difference between values obtained after 20 minutes and after 10 minutes of incubation were used for final calculations. A reagent blank containing water instead of homogenate was run concurrently. Protein was determined in an aliquot of tissue homogenate according to the procedure of Lowry and collaborators (16).

Results and discussion. Fig. 1 shows that phosphorylase activity may be measured in the liver of the chick embryo as early as the 8th day of incubation and that its concentration remains relatively constant up to day 16, after which enzyme activity rises abruptly to values similar to those found in the liver of the newborn chick and of the adult fowl. On the 14th day, a slight rise in enzyme activity was noted. A similar biphasic behavior has been observed for other enzymes in chick embryo tissues(17-19) in contrast with the linear increase of phosphorylase and UDPG-glycogen synthetase noted in rat and guinea pig embryos(4,6,20). It should be pointed out that a sharp drop in glycogen content occurs in the liver of the chick embryo on or about the 16th day of development(2), at a time when UDPG-glycogen transferase activity also declines. These results are in agreement with the results of histochemical studies(11), and with the generally accepted view that *in vivo* phosphorylase presides over glycogen breakdown and not over glycogen synthesis.

Summary. Phosphorylase activity was measured in the liver of chick embryos at various stages of development. Enzyme activity increases abruptly between the 16th and 18th day of incubation, at a time when, according to previous studies, hepatic glycogen concentration and UDPG-glycogen transferase activity decrease to relatively low values.

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Distribution of Antibody Plaque Forming Cells in Various Tissues of Several Strains of Mice Injected with Sheep Erythrocytes.* (29628)

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The localized hemolysin ("antibody plaque") technique in agar gel is a rapid and relatively simple procedure for enumerating the number of antibody forming cells in a mixed population of cells(1). This procedure has been utilized by Jerne(1,2) to study hemolysin formation *in vitro* by spleen and lymph node cell suspensions from rabbits and mice injected with red cell antigens. Ingraham and Bussard have independently described a relatively similar localized hemolysin procedure whereby immunologically competent cells from spleens of immunized rabbits are suspended in tissue culture medium containing erythrocytes and guinea pig complement and "thickened" with carboxymethoxycellulose(3). These methods lend themselves well to the study of various aspects of antibody formation, including induction period(2,3,4), effect of immunosuppressive drugs(2,3,5) and X-irradiation(5), and role of subcellular fractions, including RNA, and ribosomes(5,6) in antibody synthesis. During several studies on antibody formation using the plaque technique(5), it was noted that various lymphoid tissues from mice injected by several routes with mammalian or avian erythrocytes were not equally efficient in plaque formation. Similarly, markedly different results were observed using different strains of inbred mice. This report is concerned with the estimation of the number of antibody plaque forming cells in various tissue suspensions from several strains of mice following primary immunization with sheep erythrocytes, using the agar plaque method of Jerne. The results indicate that there may be a marked variation in the number of plaque forming cells per given cell suspension according to strain of mouse used and whether or not lymphoid or non-lymphoid tissue is tested.

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Methods and materials. Animals. The following strains of mice were used: C_3HBL, C_{57}BL, AKR, and NIH. The mice were obtained either from Jackson Laboratory, Bar Harbor, Me., or on occasion, from a local dealer. The animals were housed in groups of 6 in plastic cages containing wood shavings. They were fed Purina mouse food and water *ad libitum.*

Immunization. Groups of 6 to 10 mice