groups, the ambient temperatures were the same in both periods. In these groups, the data show that regardless of duration, antibody response was poor or non-existent at 25° and good at 35° with some variation among individual animals. After 7 days at 40°, the response was similar to that at 35°, but after 28 days at 40°, titers were generally lower than in the comparable 35° group.

In certain experiments, animals were immunized at one temperature, then transferred to another temperature during the post-immunization period. When Groups D and E were immunized at 25° and then shifted to 35°, antibody response was enhanced; conversely, when animals were immunized at 35° and then moved to 25°, the response was inhibited. This effect was particularly striking in Group N after 14 days.

The results with Groups F, G, P and Q suggest that within the limits of quantitation possible by the tube agglutination method, the temperature of the first post-immunization week is important in determining eventual antibody level. Even though animals are later moved to a favorable or to an unfavorable temperature, the eventual titer reached is strongly influenced by the temperature of the first post-immunization week.

These results indicate that it is possible to regulate antibody synthesis in poikilothermic vertebrates by varying ambient temperature. These and earlier experiments(1) also demonstrate for the first time synthesis of agglutinating antibody by a member of the class Reptilia. Other studies with lower vertebrates have shown antibody formation in teleost fishes(2-4), and amphibia(4-6), but not by cyclostomes(3). Experiments to be presented elsewhere show that antibodies of *D. dorsalis* are located in an electrophoretic zone comparable to the β_2 and γ-globulin areas of higher vertebrates.

Summary. The antibody response of the reptile, *Dipsosaurus dorsalis,* to typhoid vaccine was compared at constant ambient temperatures of 25°, 35° and 40°. Antibody response was poor or non-existent at 25°, good at 35° and moderately good at 40°. Variations in titer were noted among different individuals within a group. When animals immunized at 35° were transferred to 25°, antibody response was inhibited; in the reverse situation, the response was enhanced. Data obtained during this investigation have shown that a member of the phylogenetic class Reptilia can synthesize agglutinating antibodies and that the process can be regulated by varying the ambient temperature.

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Developmental Changes in Glycogen Content of Primordial Germ Cells in Chick Embryo.* (28097)

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The discovery of a high alkaline phosphatase content in the primordial germ cells (PGCs) of the mouse embryo(1) provided an excellent method for studying the origin and migration of these cells in mammals. Meyer(2) observed a similar phenomenon in the PGCs of chick embryos, but the substance was glycogen instead of alkaline phosphatase. This finding in the chick embryo

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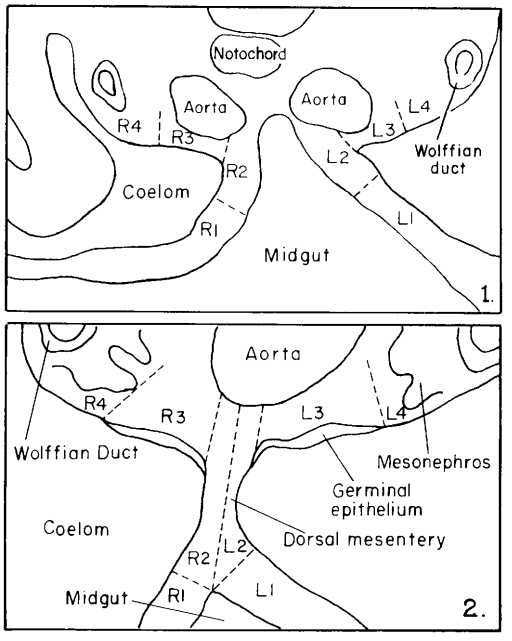

Camera lucida drawings of the area just posterior to omphalomesenteric arteries to show tPGC containing regions.

FIG. 1. Stage 16 (2 days).
FIG. 2. Stage 21 (3 days).

has provided a means for studying the mode of transport of these cells in the circulation and their migration through the tissues. Meyer(2) traced these cells through the 17th stage (52-64 hours incubation) of embryonic development (Hamburger and Hamilton, 3). The present paper reports observations on these cells from the beginning of stage 15 through stage 35 (2 through 8 days of incubation).

Materials and methods. White Leghorn chick embryos were fixed in chilled Gendre fluid and stained for glycogen by the periodic acid-Schiff technic. A 0.5% malt diastase was used for the digestion of glycogen. A light hematoxylin counterstain was utilized to outline the nuclei. The observations were made on 2 series of embryos, one based on chronological, the other on structural age. The latter were staged according to the series prepared by Hamburger and Hamilton(3).

To make differential cell counts of tissue PGCs the region containing these cells was divided into specific areas (Fig. 1 and 2). Counts were made in each area and it was noted whether the cells were present in the epithelium or the stroma of the area.

Results. Tissue primordial germ cells (tPGCs). During stage 15 (2 days) tPGCs were seen in the splanchnic mesoderm of the hypomere posterior to the origin of the omphalomesenteric arteries (Fig. 6). These cells appeared singly. Clusters were rare prior to the 4th day of incubation (stage 23) (Fig. 7). From stages 15 through 21 (3rd day) the number of tPGCs increased greatly (Table I). At stage 16 the majority of the tPGCs were located at the coelomic angle while during stage 17 (3 days) the majority were seen in the epithelium ventro-lateral to the aorta (gonadal site). This continued to be the pattern prior to the formation of the gonad from the ventro-lateral area (area 3, Table I), except for an increased number of tPGCs in the stroma beneath the aorta and in the dorsal mesentery during stage 21 (Fig. 8). By stage 25 (4 days) relatively few complete tPGCs were seen in this area but large numbers of degenerate ones were present (Fig. 9).

Cells were seen migrating into the developing gonad during the 4th day of incubation (stages 22 through 25). The cells present in the mesentery were located at or in the epithelial border with a concentration at the coelomic angle. During the 4th day clusters of 2 to 7 tPGCs were seen in the mesentery. Mitotic figures were not observed in these clusters or in any of the tPGCs until they were located in the germinal epithelium of the gonad. At 5 and 6 days some tPGCs containing glycogen could still be seen migrating into the gonad (Fig. 10).

During the 4th day (stage 25) some of these cells located in the germinal epithelium of the developing gonad, that is, having completed the migratory phase of development, had lost their glycogen. A few of them contained mitotic figures (Fig. 11). This loss of glycogen was more noticeable during the 5th day. The tPGCs still in the mesentery had retained their glycogen and were generally arranged in clusters. Those cells which occurred singly and contained glycogen were located at the point of attachment of the gonad to the mesentery or very close to it at

the edge of the mesentery. The tPGCs in the gonad near this attachment also generally retained their glycogen. By the 7th day (stage 32) most of the PGCs located in the

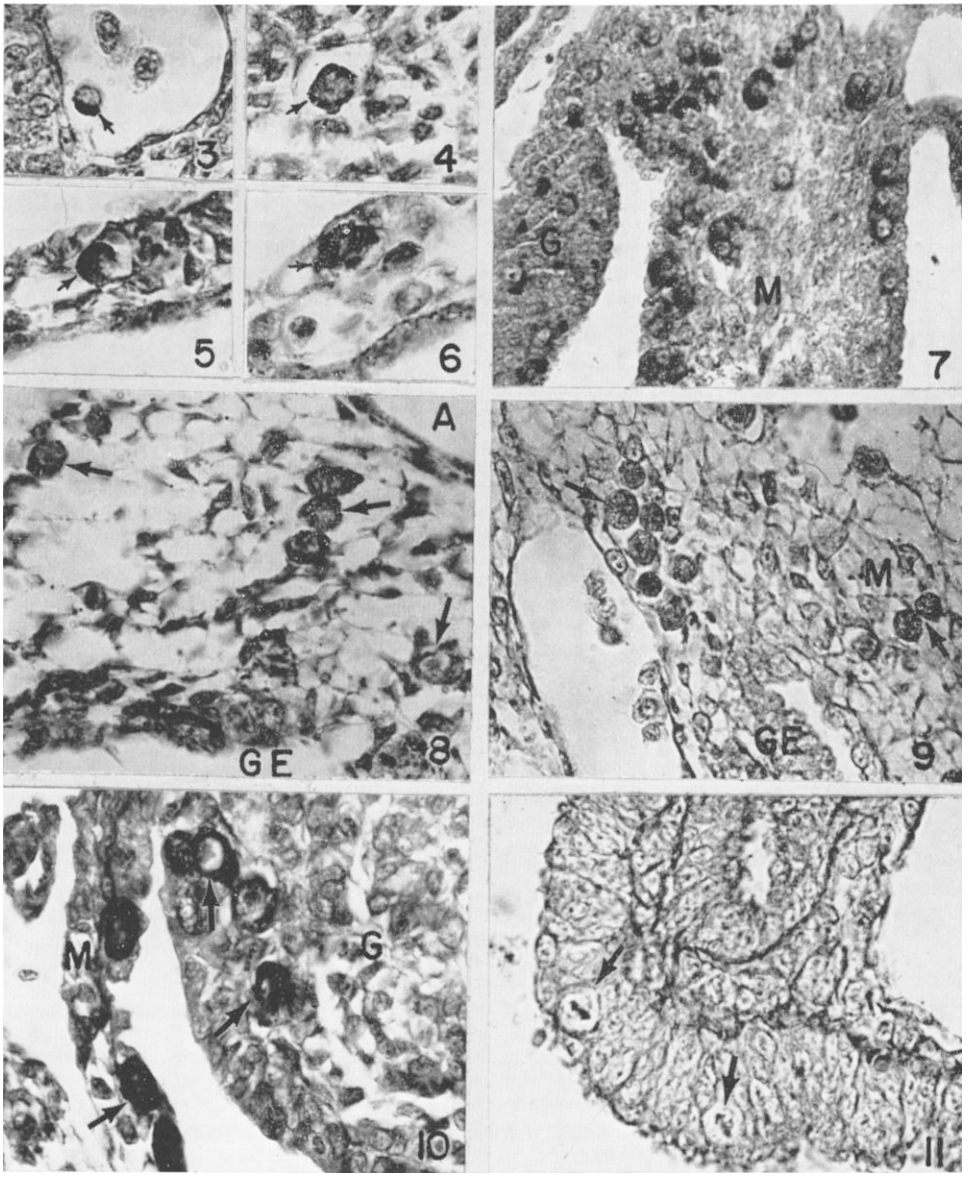

FIG. 3. Small PGC (arrow) with 2 erythrocytes in blood vessel located in area L4 (Fig. 1 and 2), (Stage 19; 78 hr). ×400.

FIG. 4. PGC in capillary in area L1 (Fig. 1 and 2), (Stage 20; 78 hr). ×400.

FIG. 5. PGC in capillary in area R1 (Fig. 1 and 2), (Stage 17; 78 hr). ×400.

FIG. 6. tPGC in hypomere area R1 (Fig. 1 and 2), (Stage 15; 66 hr). ×400.

FIG. 7. Distribution of tPGCs in mesentery (M) and gonad (G) (4 days). ×200.

FIG. 8. PGCs between dorsal aorta (A) and germinal epithelium (GE) (Stage 21; 84 hr). ×400.

FIG. 9. Degenerating PGCs located just above attachment of dorsal mesentery (M) and germinal epithelium (GE) (Stage 25; 114 hr). ×400.

FIG. 10. PGCs migrating into the gonad (G) from dorsal mesentery (M) (Stage 31; 150 hr). ×400.

FIG. 11. Dividing PGCs in germinal epithelium of gonad. Note these cells are without glycogen (Stage 25; 102 hr). ×400.

TABLE I. Differential Cell Counts of tPGCs Containing Glycogen Taken From Staged Embryos.

	Stages								
Area*	15	16	17	18	19	21	25	29	32
R1E	—	—	0	0	2	0	0	—	—
R1S	19	3	4	1	1	0	0	—	—
R2E	—	—	5	2	49	95	34	—	—
R2S	2	17	2	12	80	230	17	7	0
R3E	—	—	65	72	99	45	22†	17	0
R3S	0	6	30	35	18	14	0	4	1
R4E	—	—	4	0	0	0	0	—	—
R4S	0	0	6	1	0	0	0	—	—
R-Total	21	26	116	123	249	384	73	28	1
L1E	—	—	0	1	0	0	0	—	—
L1S	8	1	3	4	0	0	0	—	—
L2E	—	—	1	21	65	63	14	—	—
L2S	1	16	4	23	67	338	38	0	0
L3E	—	—	91	56	142	228	117‡	48	0
L3S	0	20	26	31	20	75	2	0	0
L4E	—	—	0	1	0	0	0	—	—
L4S	0	0	5	2	0	0	0	—	—
L-Total	9	37	130	139	249	704	171	48	0
Total	30	63	246	262	543	1088	244§	76	1

* Areas are shown in Fig. 1 and 2. Area 3 in the table represents site of the gonad primordium in earlier stages and the gonad itself in later stages.

R—right; L—left; E—epithelium; S—stroma.

Epithelium was not sufficiently developed in stages 15 and 16 to determine the presence of tPGCs, thus all cells in these 2 stages are considered as being in the stroma.

In stages 29 and 32 the gonad was formed. These counts were taken from female embryos.

The following cells were not included in the above counts:

† 2 cells without glycogen.

‡ 15 cells without glycogen of which 7 contained mitotic figures.

§ 128 degenerate tPGCs found in areas R and L2S.

gonad had lost their glycogen. In only a few embryos were cells seen in the mesentery at this stage and these were invariably arranged in clusters. The PGCs of embryos in stage 33 (8 days) and later rarely contained glycogen.

Source of tPGCs. The sole link connecting circulating PGCs (cPGCs) with tPGCs in this study was the presence of glycogen in the cytoplasm. Circulating cells designated cPGCs occurred in 3 forms. The first consisted of small cells with rounded nuclei and small amounts of cytoplasm (Fig. 3). Glycogen was seen at one side in a rather large granule in the cytoplasm of these cells. The second was a slightly larger cell containing more glycogen which was evenly distributed throughout the cytoplasm (Fig. 4 and 5). These were seen in capillaries and were thought to be about ready to migrate into the tissues. The third type was slightly larger than the second and was seen adjacent to the wall and in the lumen of the aorta. These cells generally contained only a few small granules of glycogen in their cytoplasm. The 3 types were usually absent from the circulation by the 5th day of incubation. However, circulating cells other than PGCs which contained some glycogen were present in all of the older embryos examined. These cells were irregular in shape and had very small granules of glycogen distributed throughout the cytoplasm.

The circulating PGCs ranged from 7 to 10 and the tissue PGCs from 9 to 17 μ in diameter. Mitotic figures were seen in the cPGCs but never in migrating tPGCs.

The cPGCs move into the tissues primarily through the walls of the aorta and of the capillaries. From the capillaries the great majority leave posterior to the omphalomesenteric arteries. Cells migrating through the walls of the aorta leave from 2 areas. Circulating PGCs became attached to the ventrolateral wall of the aorta in the region of the dorsal mesocardium. This region of adhering

cPGCs seems to be associated with the remains of the pronephros and the first part of the mesonephros. Proceeding posteriorly the cells gradually disappear and are absent in the region just anterior to the omphalomesenteric arteries. With the formation of the dorsal mesentery, cPGCs were seen adhering to the ventral wall of the aorta directly over this mesentery in the region of the developing gonad. In the anterior region the cPGCs were actually seen passing through the wall of the aorta. As soon as they have entered the tissues they apparently lose their glycogen since they cannot be traced more than several cell diameters away from the aorta. Although no cPGCs were actually seen passing through the wall of the aorta in the region of the gonads, they were seen on both sides of the wall and in the stroma beneath the aorta leading to the germinal epithelium.

Discussion. A question that arises from our study is whether the cells we classify as cPGCs are actually the same as the cPGCs reported by previous investigators(4,5). On the basis of size this would not appear to be the case since these reports indicate that the cPGCs and the tPGCs they observed were essentially the same size. However, cells of this size were not seen in the circulation of our embryos even in the earliest stage studied (stage 15). This conflict is possibly due to the fact that our sections were prepared to demonstrate glycogen, hence were not suitably stained to observe cytoplasmic or nuclear details. Our cPGCs were identified on the basis of their glycogen content and on what appeared to be intermediate stages in a transformation into tPGCs. It should be pointed out that the small glycogen containing cPGCs we observed resembled the thrombocytes of chick embryos described by Lucas and Jamroz(6). The cytological character of the cPGCs is being investigated.

Since mitotic figures were seen in PGCs being transported by the circulatory system as well as after they became resident in the gonads, but not in the period between these 2 stages, it would appear that they do not undergo division while actively migrating through the tissues.

It is of interest to note the large numerical increase in tPGCs in the stages just prior to formation of the gonad and to compare this with the noticeably smaller number observed after formation of the gonad (Table I). This decrease in the numbers counted after formation of the gonad is due, at least in part, to our inability to recognize them owing to the loss of glycogen. However, we believe that the majority of these cells, located outside of the gonad, degenerate and only a relatively few actually migrate into the gonad. The tPGCs that were seen migrating into the gonad after day 4 contained more glycogen than did the tPGCs prior to this time, either in the gonadal site prior to formation of the gonad, or in the dorsal mesentery. The disappearance of glycogen in the PGCs was correlated with their residence in the gonad. Since large amounts of glycogen were present only in migrating tPGCs it may tentatively be assumed that this carbohydrate serves as a source of energy during the period of migration.

Summary. Glycogen containing tissue PGCs on the 2nd day of incubation appeared first in the splanchnic mesoderm posterior to the omphalomesenteric arteries. These cells originated from smaller circulating PGCs which contained less glycogen. The PGCs moved out of the capillaries, enlarging and accumulating glycogen as they did so, and migrated to the gonadal site. As these cells became located in the developing gonad they lost their glycogen. This loss of glycogen became evident on the 4th day, was marked on the 5th and by the 7th the majority were without glycogen.

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