els were significantly lower with the "Italian" type diets. In subsequent studies Keys *et al.* (12) reported that citrus pectin when fed at a level of 15 g per day caused a slight but statistically significant reduction in serum cholesterol levels in physically healthy, middle-aged men; cellulose (fiber) fed under comparable conditions was without significant effect. These studies suggest that cellulose or fiber *per se* was not responsible for the low serum cholesterol levels of the Bantu and comparable groups, but that pectin, another complex bulk-forming carbohydrate, may have had some activity in this regard.‖ An analysis of the diets of native populations with low serum cholesterol levels indicated that in addition to pectin such rations also contain gums and/or other complex carbohydrates such as colloids of marine plants which were found in the present experiment to cause a highly significant reduction in liver cholesterol levels in the cholesterol-fed rat. Further studies are indicated to determine what effect these substances might have, when administered alone or in combination with one another and pectin, in treatment of hypercholesterolemia and atherosclerosis in man.

Summary. The increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the rat was largely counteracted by concurrent feeding of Gum Guar, Locust Bean Gum or carrageenan at a 10% level in the diet. Effects were similar to, although slightly less marked, than that obtained with a comparable amount of pectin N.F.

‖ Unpublished studies from this laboratory indicate that different batches of pectin may vary markedly in anti-cholesterol activity. Pectic preparations with a methoxyl content of 5% or less were without activity in counteracting the increment in plasma and liver cholesterol levels induced by cholesterol feeding in the rat in contrast to the marked activity exhibited by pectin N.F. preparations of relatively high methoxyl content (10.7% on a moisture-ash-free basis).

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Sequential Alterations of Lactic Dehydrogenase Isozymes During Embryonic Development and in Tissue Culture.* (27586)

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Enzymatic changes during growth and development have been extensively studied. The early chick embryo contains low phosphatase activity(1) relatively high peptidase activity(2) and very high concentrations of cytochrome oxidase and succinoxidase(3). During fetal development sequential alterations of phenylalanine hydroxylase, tyrosine transaminase and phenylalanine transaminase in rabbits(4) and of arginase in chicks(5), have been described, and each of these enzymes exhibits a characteristic pattern of

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changing activity. If changes in enzymatic activities reflect cellular metabolic changes, studies of fetal enzymatic alterations might provide clues to cellular development and specialization. A contrasting situation exists in cells grown in tissue culture where the relatively constant environment might be expected to discourage cellular specialization. In the present study isozymes, multiple forms of an enzyme exhibiting similar substrate specificities(6), were studied in these two differing environments, *i.e., in vivo* and *in vitro*. It was hoped that isozymes might provide more information than an enzyme present in only one molecular form. Previously isozymes have been employed in studies of differences between fetal and adult guinea pig, mouse and pig tissues(6,7).

The results of the present study show that during fetal development of the chick the activity of anodal migrating lactic dehydrogenase (LDH) isozymes decreases as the activity of the cathodal isozymes increases. In chick embryo cells grown in culture an isozyme pattern identical in all chick tissues develops within a few days, and the intensity of the anodal bands decreases with relatively increasing intensity of the cathodal bands.

Materials and methods. Fetal chick liver, skeletal muscle and heart were dissected and the isozyme pattern was examined at regular intervals following fertilization. The tissues were disrupted by sonic oscillation in phosphate buffer pH 7.0 and were centrifuged at 1500 *g* for 2 hours. The supernatant was subjected to vertical starch gel electrophoresis according to the method of Smithies(8). A phosphate citric acid buffer, 0.01 M, pH 7.0, was employed; the molarity of the solutions in proximal and distal vessels was 0.2. A potential gradient of 6V per cm was employed for 12 hours at 21°C. The gel was then cooled and sliced into 2 half-sections. The bottom half was stained for LDH activity utilizing sodium lactate as substrate, DPN as coenzyme and phenazine methosulfate as electron transporter. The dye was a tetrazolium salt nitro-BT which when reduced to the formazan produces a brilliant purple color(9, 10). In several experiments the total LDH activity of chick tissues was assayed spectrophotometrically(11) so that all samples on a given gel had a similar activity.

Trypsinized, filtered cell suspensions or cells derived from explants of various chick tissues were grown in culture bottles in reinforced Eagle's medium with 10% fetal calf serum(12). When a monolayer of cells was seen microscopically to cover the bottom of the culture bottles, subculturing was carried out by freeing and removing the cells from the bottom of the culture bottles with 0.25% trypsin solution. Cells were finally harvested, centrifuged, resuspensed in saline and thoroughly washed 3 times with phosphate buffered saline, pH 7.4, or removed from the bottle with a rubber policeman, suspended in saline, and centrifuged.

Results. Supernatants containing high concentrations (140,000 units/ml) of liver LDH from 10-day-old embryos revealed 3 major anodal bands, 2 additional rapidly migrating anodal bands of lower concentration, and 2 very lightly staining cathodal bands. By employing high LDH concentrations of adult heart (240,000) 3 anodal and 2 faint cathodal bands could be observed. These cathodal bands are not seen in the concentrations usually used in this study.

Fig. 1 illustrates the pattern obtained from fetal chick liver, muscle and heart isozymes

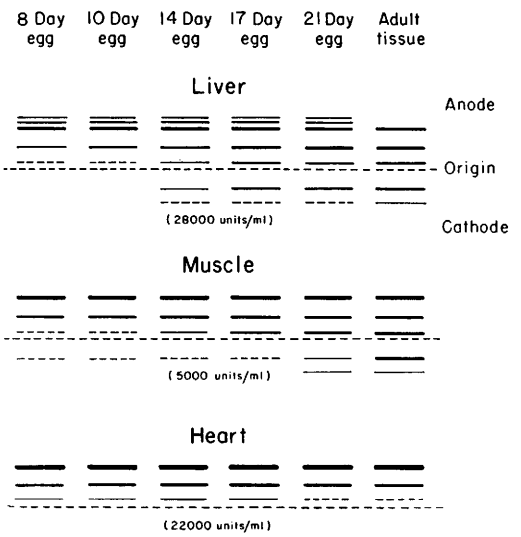

FIG. 1. Diagram of intensity, size and localization of LDH isozymes from chick tissues obtained at various days after fertilization and separated by starch gel electrophoresis.

containing 28,000, 5,000 and 22,000 units of activity per ml respectively during various stages of development beginning 8 days after fertilization. Alterations in isozyme pattern during develpment of chick liver and muscle are characterized mainly by a redistribution of total LDH activity from a heavy concentration of the anodal bands in the early embryonic tissues to prominence of the cathodal bands in later stages (Fig. 1). Heart on the other hand shows no major redistribution of LDH activity, although the slowest migrating anodal isozyme becomes less prominent with increasing age (Fig. 1).

After several days in culture, liver from 8-day-old chick, skin, muscle and heart from 10-day-old chick, muscle and heart from 21-day-old chick all assume a common LDH isozyme pattern (Fig. 2). This pattern represents a redistribution of LDH activity among the isozymes which differs radically from the isozyme pattern of the tissue of origin where the anodal bands are prominent (Fig. 1). The LDH isozyme pattern in culture is characterized by 1 or 2 heavily staining cathodal bands and 1 or 2 lightly stained anodal bands

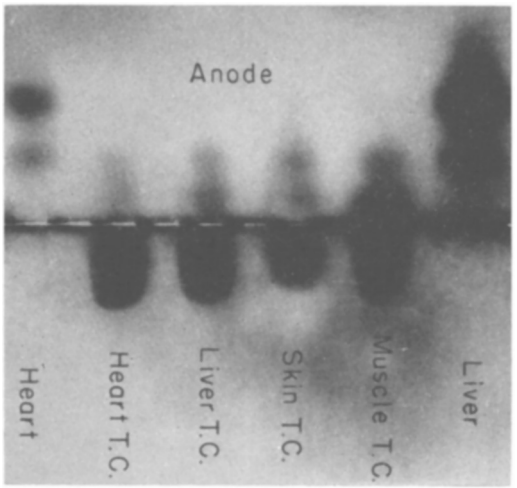

FIG. 2. A starch gel showing from left to right LDH isozymes of 10-day chick heart, 10-day chick heart in tissue culture 28 days, 10-day chick liver in tissue culture 27 days, 10-day chick skin in tissue culture 16 days, 10-day chick muscle in tissue culture 15 days and 10-day chick liver. LDH activities were not quantitated. Note similarity of isozyme patterns of 4 tissues in culture and difference of this pattern from that of tissue of origin.

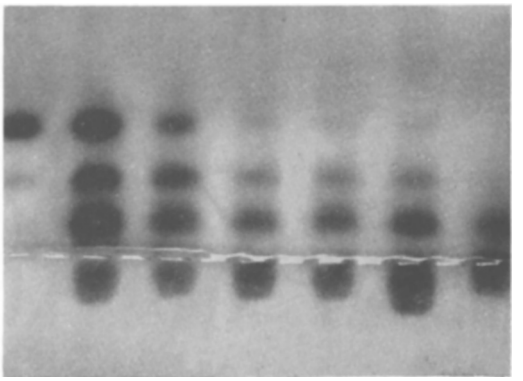

FIG. 3. A starch gel showing from left to right LDH isozymes of 10-day chick muscle after trypsinization, 10-day chick muscle in tissue culture 2 days, 4 days, 6 days, 8 days, 10 days and 16 days. All tissue culture material applied to the gel contained approximately 20,000 units of LDH activity/ml.

(Fig. 2). Sequential alterations of the LDH isozyme pattern from a single tissue in culture occur with time and consist of a redistribution of activity characterized by a decreased activity in the anodal bands (Fig. 3).

Discussion. These data on chick tissues during fetal development and in culture indicate in both systems the occurrence of sequential alterations consisting of a redistribution of total LDH activity among the isozymes. With time there is a decrease in intensity of the anodal isozymes and a relative increase in intensity of cathodal isozymes. The observation that the chick tissues assume a characteristic isozyme pattern under the conditions of tissue culture employed in these experiments (Fig. 2 and 3) suggests that the environment in culture favors the development of a common cell type. Under other *in vitro* conditions, the isozyme pattern of the tissue of origin may be more closely retained.

It may be concluded that isozymes provide a useful method for showing cellular alterations *in vitro* as well as *in vivo*. They may also be employed to guide experiments directed at changing conditions of tissue culture in order to preserve the normal physiological activities of cells grown *in vitro*. The importance of environmental factors under *in vitro* conditions is emphasized by several studies. By appropriate changes in the culture medium it has been demonstarted that cells with

various morphologies could be favored(13, 14) and also that enzymes could be induced (15). Levintow and Eagle state that with quantitative studies of enzymes the activities in primary cultures generally reflect those of the tissue of origin, but as the cells proliferate *in vitro* the organ-specific enzyme activities diminish until a "neutral" uniform level characteristic of serial cultures emerges(16). Although galactosemic and glucose-6-phosphate dehydrogenase-deficient cells have been successfully maintained in culture(17,18), Lieberman and Ove have demonstrated that various types of human cells with different *in vivo* enzymatic activities assume common enzymatic activities in culture(19). Several possible explanations have been suggested to account for these observations. Selection of a cell type favored under the cultural conditions employed or a continuous selection of similar mutants from different cells may occur. Alternatively, the common enzyme pattern may arise from different cells which undergo a similar "dedifferentiation" in culture where the environment fails to sustain the *in vivo* interplay between various cell types(5). The present study suggests that comparative observations on the isozyme patterns of various enzymes *in vivo* and *in vitro* may help elucidate this problem.

Summary. 1. During development fetal chick liver and muscle show sequential alterations in distribution of LDH isozymes. These changes are characterized by a progressive increase in concentration of cathodal bands relative to anodal bands. In chick heart the LDH isozyme pattern remains relatively constant during development. The number of bands depends on the activity applied to the gel, and with high LDH activity chick heart, muscle and liver 10 days after fertilization show the full tissue complement of LDH isozymes. 2. The chick embryo tissues studied in tissue culture reveal under the conditions employed in this study a common pattern characterized by 1 or 2 heavily stained cathodal bands and 1 or 2 lightly stained anodal bands. During prolonged culture the anodal bands decrease in concentration. Isozymes may prove to be a sensitive index of the maintenance of physiological activities of cells grown in tissue culture.

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