AN ANALYSIS OF THE SYNTHESIS AND DISTRIBUTION OF THE CONTRACTILE PROTEIN, MYOSIN, IN THE DEVELOPMENT OF THE HEART

BY JAMES D. EBERT

INDIANA UNIVERSITY*

Communicated by B. H. Willier, February 10, 1953

The development of the heart presents a series of problems in morphogenesis and differentiation which afford an unexcelled opportunity for the analysis of the interrelationships of specific structural and functional properties during ontogeny. As the heart is formed, so it begins to beat. In the chick embryo of 7 somites the paired primordia of the heart have already begun to fuse; local contractions have been recorded in the heart of the 9-somite embryo.[1, 2] The onset of contractility has been correlated with the time at which significant histogenic changes have been recorded, e.g., the first appearance of cross-striations at the 10-somite stage[3] and the first detectable glycogen in the embryo of "about 28 hours."[4] The early contractions have been described as of a fundamental myogenic nature since they occur well in advance of the time at which the first nerve fibers approach the heart.

The early morphogenesis and onset of contractility of the heart are foreshadowed by a precocious restriction of the heart-forming cells to relatively well-defined areas of the blastoderm. Willier and Rawles[5] and Rawles[6] have described two large bilaterally situated areas of the head-process blastoderm endowed with heart-forming potency. No critical information has been presented concerning the location of the heart-forming areas earlier than the head-fold stage. Rudnick[7] has concluded from a review of the pertinent data that the heart mesoderm must be very early in a highly organized state, and is early invaginated to migrate laterally to its definitive position. A similar conclusion was reached by Bacon[8] in his analysis of the development of the heart in *Amblystoma*.

In contrast to the fund of information from the field of experimental morphogenesis, information concerning the chemistry and cellular physiology of the development of the heart is almost completely lacking. The void may be due, at least in part, to the difficulties encountered in studying the contractile proteins in minute quantities, as illustrated by the experiences of Herrmann and his coworkers,[9, 10] who have not been able to make determinations of actomyosin in skeletal muscle of the chick embryo prior to the ninth day of incubation, although fibrillations and cross-striations have been observed in the 7-day embryo, a time at which muscular response to mechanical stimulation and histological differentiation of motor centers in the central nervous system also have been described. In the

present investigation the difficulty in detecting minute quantities of cardiac contractile proteins is largely overcome through the application of the precise techniques of immunochemistry.

In earlier reports[11, 12] it was shown, by absorption and precipitin tests, that antisera produced in rabbits against extracts of adult chicken heart will react with extracts of the early chick embryo prior to the morphogenesis of the heart. The early blastoderm must therefore contain antigens identical, or closely related, to those of adult heart. This conclusion is supported by the finding that when early blastoderms are cultured in media containing antiheart sera, the development of the heart is differentially suppressed.

It becomes of interest to determine next the relationship of the specific antigenic components of cardiac muscle present in the early blastoderm to the contractile proteins, actin and myosin. We ask the following questions: (1) Do actin and myosin develop simultaneously? Is the development of each protein an independent event or does the development of one precede that of the second and have a causal role in its synthesis?

(2) Are there differences in the contractile proteins of cardiac and skeletal muscle which play a significant role in the precocious restriction of cardiac contractile proteins to the heart-forming areas of the blastoderm, and in the early onset of contractility? Only after the facts relative to the time of synthesis and distribution of the cardiac contractile proteins are available will it be possible to attack the basic problem of the factors controlling their synthesis. The specific objective of this report is to present findings relative to the time of synthesis and progressive localization of cardiac myosin in the early chick embryo.

METHODS AND RESULTS

1. Preparation of Myosin.—Cardiac and skeletal myosins were extracted from heart and hind limb muscle, respectively, of adult New Hampshire Red chickens of both sexes, following the method described by Mommaerts and Parrish,[13] in which myosin and actomyosin are simultaneously precipitated from the muscle extract by dialysis, most of the actomyosin being irreversibly insoluble. The remaining actomyosin is precipitated from the redissolved myosin by adjusting the ionic strength and pH. Actin-free myosin is then crystallized by dilution with glass-distilled water. Twelve to 36 fresh adult chicken hearts were used in each extraction (100 to 350 g. of fresh cardiac muscle). Two minor deviations from the technique described by Mommaerts and Parrish should be noted: (1) following dialysis for 18 hours, the ionic concentration of the muscle extracts is frequently not reduced sufficiently to permit maximal precipitation; therefore, 200 ml. of glass-distilled water per 100 g. of muscle is added directly to the extract following dialysis. (2) Mommaerts and Parrish

describe an average yield of 1.2 g. of myosin from 100 g. of muscle. Our yields have been consistently lower: from chicken cardiac muscle, 750 mg. of myosin per 100 g., and from chicken skeletal muscle, 410 mg. of myosin per 100 g. of muscle extracted.

It must be emphasized at the outset of the discussion that although the extraction technique employed yields actin-free myosin which approaches purity, the final product is not monodisperse. Mommaerts and Parrish state that the purity of their preparations is of the order of 85–90 per cent. Preliminary studies of cardiac myosin indicate that the degree of purity of the preparation is only slightly lower than that described by Mommaerts and Parrish.

2. Production of Antisera.—Despite the brief positive report by Kesztyus, Nikodemusz, and Szilagyi[14] that myosin is antigenic, our early attempts to produce potent antisera in rabbits against cardiac myosin from adult chickens were unrewarding.[12] Hence it seems advisable to record here, briefly, the more important details of the immunization procedure which has proved to be reliable in our laboratory during the past year. First, it is essential that the myosin used for injections be either freshly prepared or stored for less than one week at −20°C. In the latter case, it should be thawed only once. The use of freshly extracted myosin for each injection is prohibitively expensive of both time and materials, hence we have made extractions weekly. The myosin should be a bluish-translucent gel; no shreds are desired. The myosin is stored and injected in the phosphate-KCl solution described by Mommaerts and Parrish, at pH 6.8, ionic strength 0.5.

Individual rabbits vary greatly in their antibody response to injected myosin. To date, of 22 rabbits injected with cardiac myosin, only 12 have produced antisera of sufficient potency to be of value. In general, the immunization schedules are so arranged that each adult male rabbit receives a series of intravenous injections on alternate days over a two-week period, totaling 18–20 mg. of protein, is rested for 4–6 days and bled. After 20–25 days further rest, a second series of injections is started, in which a first injection of 4–6 mg. of protein is given intraperitoneally, followed by a total of 48–60 mg., intravenously, on alternate days over a two-week period. Once again the rabbits are bled following 4–6 days rest. Our rabbits have consistently built up circulating antibodies against myosin rapidly, and lost them rapidly. Hence the rabbits are bled 4–6 days rather than the more general 7–10 days after the last injection of a series. Sera are sterilized by passage through a Seitz filter and stored at −20°C. No preservative is added.

3. Specificity of Antisera.—The titer and specificity of each serum was determined by the precipitin technique, employing homologous or heterologous antigen. A preliminary titration was made with a constant

volume of full-strength serum and progressively decreasing quantities of homologous antigen. The end-point of the antigen dilution series was then titrated with serial dilutions of serum. In this manner, inhibition due to excess antigen is avoided, the serum end-point being greatest with the last active dilution of antigen.[15-17] A total of 54 antisera from 22 rabbits has been assayed. Appropriate controls have been carried out with sera of uninjected rabbits. The effective serum dilution titers of the antisera tested ranged from 1:64 to 1:8192. Only those sera with titers of at least 1:512 with homologous antigen were retained (31 antisera). Potent sera were then tested for reactivity with heterologous antigen (skeletal myosin). The results clearly show that the antisera against cardiac myosin are highly tissue-specific, i.e., that cardiac and skeletal myosins differ markedly in antigenic constitution. The highest titer obtained in tests of 27 antisera against cardiac myosin with nine separate fresh preparations of the heterologous antigen, skeletal myosin, is 1:256. There is a consistent difference of 4–5 tubes in the reactivity of each serum with homologous and heterologous antigen. In further tests, antisera were first completely absorbed with adult skeletal myosin, and then tested with homologous antigen. Much of the specific reactivity of the sera was retained. The reduction in reactivity due to elimination of antibodies to reactive groups in the heterologous antigen ranged from 1 to 4 tubes. Therefore, due to the marked difference in antigenic constitution of cardiac and skeletal myosins, it is possible to prepare, by absorption procedures, antisera highly specific for adult cardiac myosin. In the sections to follow, all references to antisera against adult cardiac myosin refer to these highly specific, absorbed antisera.

4. *Myosin in the Developing Heart.*—It will be of interest to examine next the evidence pertaining to the antigenic specificity of cardiac myosin during development, as determined by an analysis of the reactivity of antisera against adult cardiac myosin with cardiac extracts from embryos of 36 hours to 18 days incubation. All embryos used in these experiments were of the New Hampshire Red breed, from eggs incubated at 37.5°C. After removing the embryos from the eggs as quickly as possible, the hearts were removed (in an iced Petri dish), freed from excess blood with filter paper, weighed rapidly on a micro-torsion balance and minced finely with scissors on chilled waxed paper. The mince was homogenized in a Ten Brock glass tissue grinder in buffered KCl solution. At 18 days, 60 hearts, and at 13 days, 144 hearts were extracted by the technique described for adult cardiac muscle, with a workable yield of purified myosin. At the earlier stages, however, it was necessary to employ a crude extract of cardiac muscle (containing both myosin and actomyosin), since a measurable yield of purified myosin could not be obtained from the small quantities of material available. Hearts of embryos of from 36 hours to 12 days in-

cubation were homogenized in the buffered KCl solution. The homogenate was clarified by filtration through cheesecloth followed by centrifugation at 3600 r. p. m. for 30 minutes at 1°C. Both the purified cardiac myosin from older embryos and the clarified cardiac extract from younger embryos were tested for reactivity with antisera against adult cardiac myosin employing several modifications of the precipitin technique, including (1) the ring test, (2) the Hanks technique,[15, 18] and (3) the micro (capillary) method of Ten Cate and Van Doorenmaalen,[19] kindly demonstrated to me by the authors in their laboratory. All preparations from cardiac muscle, both the purified myosin and the clarified extract from younger embryos, reacted positively with all antisera tested. Cardiac extracts and purified cardiac myosin from embryos at the following stages have been tested: 36, 48, and 72 hours, 5, 7, 9, 12, 13, 15, and 18 days. At least three separate fresh extracts have been tested at each stage, each extract being reacted with at least three potent anticardiac myosin sera. Further, by the absorption technique, employing repeated absorptions with em-

TABLE 1

CARDIAC MYOSIN IN THE EARLY EMBRYO

HAMBURGER AND HAMILTON STAGE	TOTAL NUMBER OF EMBRYOS EXTRACTED	NUMBER OF FRESH EXTRACTS TESTED	NUMBER OF ANTISERA USED WITH EACH EXTRACT	TOTAL NUMBER OF TEST SERIES	NUMBER OF POSITIVE REACTIONS	HIGHEST TITER
1	264	5	3	15	0	...
2	248	5	3 or 4	18	1	4
3	236	5	3	15	15	256
4	381	7	2 or 3	17	17	256
5	140	3	2 or 3	8	8	512

bryonic cardiac antigens of any of the stages tested, it has been clearly demonstrated that all activity of the antisera for adult cardiac myosin can be removed. It is concluded, therefore, that the embryonic heart, from 36 hours through 18 days incubation, contains antigens or reactive groups identical, or closely related to those found in adult cardiac myosin.

5. *Cardiac Myosin in the Embryo Prior to Morphogenesis of the Heart.*— The question next arises as to the time at which the reactive groups of cardiac myosin first can be detected in the early embryo. In this series of experiments, embryos in stages 1 to 5 of the Hamburger-Hamilton series (pre-streak through head-process stages) were extracted following the method described for the extraction of cardiac tissue of embryos from 36 hours to 12 days incubation. Three dozen or more embryos at each stage were rapidly removed from the yolk, trimmed and washed to remove adhering yolk, and homogenized in ice-cold KCl-phosphate solution in the Ten Brock grinder. The homogenate was allowed to stand for 1 hour at 2°C., with constant stirring, and then clarified by filtration through cheesecloth, followed by centrifugation. The resultant clear extract was

used as the test antigen in precipitin tests with antisera against adult cardiac myosin, chiefly by the Hanks' and capillary methods. The results, which are summarized in table 1, are based on 73 separate serum-dilution series of reactions, for which a total of 1269 embryos has been extracted. It is clear that reactive groups identical with or closely related to cardiac myosin first can be detected in the embryo by the methods employed at stage 3, the mid-streak blastoderm. All tests of pre-streak, and 17 out of 18 tests of initial streak embryos were negative. At the latter stage one exception was noted—a trace reaction in the first 2 tubes of 1 series. The experiment was repeated with 3 additional fresh extracts of embryos at the same stage; all were completely negative with the same antiserum; therefore the trace reaction is not regarded as significant, likely being due to an error in staging one or more embryos in the extract in question.

6. Distribution of Cardiac Myosin in the Early Embryo.—Rawles[6] has

TABLE 2

ANTERO-POSTERIOR DISTRIBUTION OF CARDIAC MYOSIN

HAMBURGER AND HAMILTON STAGE	TOTAL NUMBER OF EMBRYOS EXTRACTED	NUMBER OF FRESH EXTRACTS TESTED	NUMBER OF ANTISERA USED WITH EACH EXTRACT	TOTAL NUMBER OF TEST SERIES	SECTION OF EMBRYO TESTED	NUMBER OF POSITIVE REACTIONS	HIGHEST TITER
4	601	11	2–4	29	Ant.	29	64
					Mid (node)	29	256
					Post.	29	256
5	664	12	2–4	31	Ant.	0	...
					Mid (node)	31	256
					Post.	2	4

mapped rather precisely the shape, average dimensions and position of the heart-forming areas in the chick blastoderm at the head process stage. Her cogent analysis has provided the experimental target for the present investigation. Attention was directed first to a study of the distribution of cardiac myosin in the antero-posterior axis of the embryo at stages 4 and 5. The blastoderm at stage 4 or 5 was divided into 3 segments, designated anterior, mid (containing the node), and posterior, by 2 transverse cuts made with glass or freshly sharpened steel needles across the blastoderm at the levels defined by Rawles as the anterior and posterior limits of the heart-forming areas, 0.6 mm. in front of, and 0.45 mm. behind, the node, respectively. Anterior, mid, and posterior segments, respectively, were pooled in cold buffered KCl solution and extracted following the procedure described for whole embryos. The clarified extracts were tested for the presence of reactive groups of cardiac myosin by the capillary method. The results are summarized in table 2. It is clear that in the

embryo at stage 4, cardiac myosin is found in all 3 sections of the embryo in the antero-posterior axis. The data further suggest, but do not prove, that the amount of cardiac myosin in the mid and posterior segments is greater than in the anteriormost region of the stage 4 embryo. In order to establish the observed difference as a significant one it will be necessary to so modify the present micro-techniques that strictly quantitative data can be obtained. Efforts in this direction are being continued. In the embryo at stage 5, cardiac myosin is confined to the mid segment in the antero-posterior axis, being absent from the anterior and posterior segments. Cardiac myosin is limited in its distribution in the antero-posterior axis, therefore, to a region slightly over one millimeter in length. The possible mechanism of the progressive localization of cardiac myosin will be discussed in a later section, but it should be emphasized at this point that the

TABLE 3

MEDIO-LATERAL DISTRIBUTION OF CARDIAC MYOSIN

HAMBURGER AND HAMILTON STAGE	TOTAL NUMBER OF EMBRYOS EXTRACTED	NUMBER OF FRESH EXTRACTS TESTED	NUMBER OF ANTISERA USED WITH EACH EXTRACT	TOTAL NUMBER OF TEST SERIES	SECTION OF EMBRYO TESTED	NUMBER OF POSITIVE REACTIONS	HIGHEST TITER
4	271	6	2 or 3	13	Left lat.	13	64
					Median (streak)	13	64
					Right lat.	13	64
5	339	7	2 or 3	16	Left lat.	16	64
					Median (streak)	14	8
					Right lat.	16	64
6–7	273	5	2 or 3	11	Left lat.	11	128
					Median (streak)	0	...
					Right lat.	11	128

demonstrated localization is of a specific contractile protein and not merely of prospective cardiac cells destined for the synthesis of that protein.

Next let us consider the distribution of cardiac myosin in the medio-lateral axis. Again the data of Rawles provide the morphological basis for our experiments. Groups of embryos at stages 4 and 5 were transected as previously described, 0.6 mm. before, and 0.45 mm. behind, the node. The mid-piece, however, was further subdivided into 3 sections, by 2 cuts perpendicular to its cut surfaces, 0.2 mm. on either side of (and roughly paral'el to) the node. The resultant sections, designated left lateral, median, and right lateral, respectively, were pooled and extracted, and tested for the presence of the reactive groups of cardiac myosin as previously described. The data are are summarized in table 3.

In the embryo at stage 4, cardiac myosin is approximately equally

distributed in the 3 sections tested in the medio-lateral axis, and has not been localized to the heart-forming areas. In the embryo at stage 5, although cardiac myosin is largely localized in the lateral areas, traces of the reactive groups are found in the median section (0.4 mm. wide) containing the streak. Thus the data are not in complete agreement with the finding by Rawles that the median area does not produce heart when grafted to the chorio-allantoic membrane. Although the reaction is weak, it is consistently found and is believed to be significant. It appeared likely that cardiac cells are being invaginated even as the streak regressed. In order to determine whether the localization of cardiac myosin to the prospective heart-forming areas is complete at a later stage, the experiments were extended to include stages 6–7 (stage 6 is the transitional head-fold stage, difficult to "schedule"; hence stages 6 and 7 are pooled). The results, shown in table 3, demonstrate that in the embryo at stages 6–7 the median (streak) region does not contain cardiac myosin detectable by the immunochemical methods employed.

DISCUSSION

The labile protein which has been characterized from the physicochemical point of view (at least within reasonable limits), and designated "myosin" by most workers in the field of muscle chemistry has been the myosin extracted from rabbit skeletal muscle. The further questions as to whether myosin is species- and organ-specific have received little attention. A recent study[20] of skeletal myosins prepared from tissues of the rabbit, rat, and mouse has led to the conclusion that the myosins from these species are practically indistinguishable on the basis of a comparison of electrophoretic properties, sedimentation rates, yields, viscosities, and specific refractive increments. On the other hand, the very fact that myosins have been shown to be antigenic argues for species differences of sufficient magnitude to account for their observed ability to stimulate antibody production. Kesztyus, Nikodemusz, and Szilagyi[14] report that rabbit skeletal myosin is antigenic in dogs, but that it is not isoantigenic. Our own findings show that chicken cardiac myosin is antigenic in the rabbit.

With respect to the problem of the organ-specificity of myosin, Snellman and Gelotte[21] have reported that cardiac and skeletal myosins of the rabbit show several differences, e.g., in solubility and in the sedimentation constant, and in the firmness with which actin and myosin are bound (less in cardiac than in skeletal actomyosin). The results of the present study clearly show that cardiac and skeletal myosins are markedly different in antigenic constitution, a difference which has been of importance in establishing the specificity of the antisera employed in detecting cardiac myosin in the early embryo. The question remains unanswered, however, as to whether antigenic differences reflect important physical differences in

the proteins, and is currently the objective of a comparative physical study in this laboratory.

Next let us consider the question as to whether embryonic cardiac myosin is qualitatively the same as that in the adult. On the basis of differences in behavior of actomyosins from newborn and adult animals (guinea pigs, rats, mice) upon the addition of adenosinetriphosphate, Ivanov and Kasinova[22] concluded that actomyosin in the newborn animal differs qualitatively from that in the adult. Csapo and Herrmann[10] do not support this conclusion, arguing that, at least in their comparison of chick embryonic and adult actomyosins, only quantitative changes occur. Our findings support the latter view, inasmuch as the reactive groups of cardiac myosin capable of combining with specific antisera to adult cardiac myosin do not change qualitatively from the embryo of 36 hours to the embryo of 18 days or, in fact, to the adult fowl.

It will be of interest to consider next the questions of the time of synthesis and localization of cardiac myosin in the early embryo, with particular reference to the concept of organ-forming areas. It is necessary to recognize at the outset of the discussion that while the data clearly demonstrate that a major synthesis of cardiac myosin is initiated only after the prospective mesodermal cells of the epiblast have invaginated, the evidence does not permit a discussion of the problem of mechanism of synthesis. It is clearly possible that "traces" of cardiac myosin may be present in the earlier embryo-quantities which cannot be detected even by the highly sensitive methods employed. The present findings do present a sound chemical basis for further experiments designed to test the related hypotheses of the regulation of the growth and differentiation of an organ by its own products[15, 23] and of pattern formation involving the specific suppression of embryonic differentiation by metabolites of differentiated tissues.[24] We ask, for example, whether the reported failure of cardiac tissues to differentiate in the presence of extracts or products of fully differentiated heart muscle[23, 24] results from a suppression of the synthetic activities of the tissue (e.g., in the synthesis of contractile proteins), or from a failure in morphogenesis of synthesized products.

Tests of developmental potencies of small pieces of the chick blastoderm through transplantation to the chorio-allantoic membrane clearly show that each of the isolated pieces possesses a relatively restricted developmental capacity. Particular structures develop only from rather definite areas of the blastoderm; in short, the organ-forming areas are localized to a remarkable extent. Recently, the nature and significance of the concept of the organ-forming areas has been the object of sharp criticism.[25] Yet most of the investigators who have employed this approach have been acutely aware of its major limitation, namely that each of the isolated pieces is triploblastic, and have, in fact, emphasized the importance of

tissue interactions. Moreover, it has been recognized that the degree of specificity of localization of the several organ-forming areas differs. In fact, on the basis of his recent investigation of the localization of prospective neural plate in the early chick blastoderm, in which the results obtained by transplantation and explantation are shown to be in close agreement with those obtained by marking methods, Spratt[26] has concluded that both types of methods reveal primarily the prospective fates of different regions of the blastoderm. In the case of the heart-forming areas, however, as Rudnick has emphasized,[7] the areas are amazingly large, exceeding in size the actual myocardial anlagen. Thus it appears that the heart-forming areas are areas of prospective potency and not of prospective fate.

Implicit in our definition of an organ-forming area, e.g., the heart-forming area, has been the assumption that the cells of that area of the embryo are destined to become specialized for the synthesis of the specific proteins characteristic of the definitive organ, and that the progressive localization of the heart-forming area is a localization of prospective myosin (and other cardiac protein) synthesizing cells. Yet the present findings demonstrate the progressive localization of the specific reactive groups of cardiac myosin, and not merely of prospective cardiac cells. Cardiac myosin is synthesized in the early embryo and can be detected in stage 3; in the embryo at stage 4, the protein is detected in all sections of the embryo analyzed (presumably in the mesoderm, although the present experiments do not prove this), being more concentrated in the mid and posterior, than in the anteriormost, regions. This finding is not in complete agreement with the results of explantation experiments. Spratt[27] studied the development of pulsating heart masses from anterior and posterior pieces of early blastoderms cultivated *in vitro*, and concluded that although heart-forming potency is wide-spread in pre-streak embryos (see also ref. 28), it later becomes localized in the median portion of the posterior half of the streak at stage 4. Rudnick[29, 30] likewise found that prior to its definitive localization at stage 5, the center of the heart-forming region lies posterior in the streak. The present findings do suggest that the synthesis of myosin is greater in the posterior than in the anterior part of the blastoderm; nevertheless some cardiac myosin is synthesized in the anterior part of the embryo. It is interesting to note that both Spratt and Rudnick used the classical plasma-embryo extract medium in their explantation studies, a medium which according to the reports cited above[23, 24] might suppress cardiac differentiation, since in the absence of a statement to the contrary, the embryo extract is assumed to be an extract of the whole embryo, including the heart.

In the embryo at stage 5, and even more clearly at stages 6–7, the localization of cardiac myosin is in good agreement with the data of Rawles. Cardiac myosin is progressively restricted in distribution; at stage 5 the protein is limited at least to the region bounded by the anterior and pos-

terior borders of the heart-forming areas defined by Rawles, viz., 0.6 mm. forward of, and 0.45 mm. behind, the node. The localization of cardiac myosin in the medio-lateral axis is not completed until stages 6–7, although the protein is largely confined to the lateral heart-forming areas at stage 5.

It is probable that the bulk synthesis of cardiac myosin is initiated only following the invagination of the prospective cardiac cells through the patent streak. The first condition being fulfilled, myosin is synthesized in all areas of the embryo tested.

Emphasis has already been placed on the fact that it is those cells already activately synthesizing cardiac myosin, as defined by its combining groups which are localized, and not merely those cells destined to synthesize the protein. Obviously the present findings do not fall readily into any category with respect to current theories of differentiation. In fact, no single interpretation is offered here. If it is postulated either that myosin synthesis is a self-limiting reaction or that other factors are limiting (e.g., availability of substrate, to cite one of the arguments frequently advanced), it then becomes necessary to explain the disappearance of myosin from the noncardiac areas of the blastoderm. At first thought it appears unlikely that the specificity of the protein might be altered and the protein redirected into other channels. Yet Daly and Mirsky[31] have offered evidence of the synthesis of a protein "pool" in the exocrine pancreas, a pool from which highly specific proteins are rapidly synthesized as the secretory activity of the gland demands that the supply of specific enzymes be replenished. The degree of change of specificity in these transformations is not known. It is possible that the localization of the protein is merely a sorting-out or segregation phenomenon, the result of cellular movements within the mesoderm. Our present knowledge of morphogenetic movements within the mesoderm does not permit a critical evaluation of this possibility.

The question is posed as to whether it will be possible to characterize other organ-forming areas by immunochemical or biochemical methods. Obviously an organ or tissue must fulfill two criteria for successful experimentation in this direction, both of which are admirably fulfilled in the heart-forming areas: it must be an organ which attains early morphological or functional status and for which a sound chemical basis of function has been established. Several of the organ-forming areas which fulfill the second criterion, e.g., the thyroid-forming area, fail when the first criterion is considered. In our laboratory at present one possibility is being investigated (in addition to the analysis of the synthesis of cardiac actin as related to the present study), viz., an immunochemical study of the "pigment-forming area" of the blastoderm. Further discussion and interpretation of the findings and their bearing on the significance of the concept of "organ-forming areas" is reserved pending the outcome of these experiments.

* Contribution number 521 from the Zoological Laboratories. The investigation is supported by grants from the National Science Foundation (NSF-G15) and Indiana University, and by an Indiana University Graduate School Research Fellowship. I wish to acknowledge the intellectual and material aid of the staff of the Department of Biology, Massachusetts Institute of Technology, where the investigation was initiated, and the technical assistance of Alton M. Mun and Louise A. Ross.

[1] Hamburger, V., and Hamilton, H. L., *J. Morph.*, **88**, 49–92 (1951).

[2] Patten, B. M., and Kramer, T. C., *Am. J. Anat.*, **53**, 349–375 (1933).

[3] Lewis, M. R., *Johns Hopkins Hosp. Bull.*, **30**, 176–181 (1919).

[4] Allen, H. J., *Biol. Bull.*, **36**, 63–70 (1919).

[5] Willier, B. H., and Rawles, M. E., *Proc. Soc. Exptl. Biol. Med.*, **32**, 1293–1296 (1935).

[6] Rawles, M. E., *Physiol. Zool.*, **16**, 22–42 (1943).

[7] Rudnick, D., *Quart. Rev. Biol.*, **19**, 187–212 (1944).

[8] Bacon, R. L., *J. Exptl. Zool.*, **98**, 87–125 (1945).

[9] Herrmann, H., *Ann. N. Y. Acad. Sci.*, **55**, 99–108 (1952).

[10] Csapo, A., and Herrmann, H., *Am. J. Physiol.*, **165**, 701–710 (1951).

[11] Ebert, J. D., *J. Exptl. Zool.*, **115**, 351–377 (1950).

[12] Ebert, J. D., *Ann. N. Y. Acad. Sci.*, **55**, 67–84 (1952).

[13] Mommaerts, W. F. H. M., and Parrish, R. G., *J. Biol. Chem.*, **188**, 545–552 (1951).

[14] Kesztyus, L., Nikodemusz, S., and Szilagyi, T., *Nature*, **163**, 136 (1949).

[15] Ebert, J. D., *Physiol. Zool.*, **24**, 20–41 (1951).

[16] Martin, D. S., *J. Lab. Clin. Med.*, **28**, 1477–1482 (1943).

[17] Kabat, E., and Mayer, M., *Experimental Immunochemistry*, Charles C Thomas, Springfield, Ill., 1948.

[18] Hanks, J. H., *J. Immunol.*, **28**, 95–104 (1935).

[19] Ten Cate, G., and Van Doorenmaalen, W. J., *Koninkl. Ned. Akad. Wetenschap., Proc.*, **53**, 3–18 (1950).

[20] Miller, G., Golder, R. H., Eitelman, E., and Miller, E., *Arch. Biochem. and Biophys.*, **41**, 125–137 (1952).

[21] Snellman, O., and Gelotte, B., *Exp. Cell Res.*, **1**, 234–252 (1950).

[22] Ivanov, I. I., and Kasinova, B. S., *Doklady Akad. Nauk. S. S. R.*, **60**, 417 (1948).

[23] Weiss, P., *Science*, **115**, 487–488 (1952).

[24] Rose, S. M., *Anat. Rec.*, **113**, 527 (suppl.) (1952).

[25] Waddington, C. H., *The Epigenetics of Birds*, Cambridge University Press, 1952.

[26] Spratt, N. T., Jr., *J. Exptl. Zool.*, **120**, 109–130 (1952).

[27] Spratt, N. T., Jr., *Ibid.*, **89**, 69–101 (1942).

[28] Olivo, O. M., *Compt. rend. assoc. anat.*, **23**, 357 (1928).

[29] Rudnick, D., *Anat. Rec.*, **70**, 351–368 (1938).

[30] Rudnick, D., *J. Exptl. Zool.*, **79**, 399–427 (1938).

[31] Daly, M. M., and Mirsky, A. E., *J. Gen. Physiol.*, **36**, 243–255 (1952).