The theory[10] applied in the present note has previously been used primarily in the study of sequences of functions approximating to a given analytic function, rather than in the study of the relations between various asymptotic properties of other sequences of functions. However, the method of proof of our main theorem is of wide applicability in the study of sets of functions other than polynomials, such as orthogonal functions defined by extremal properties in a region which is not necessarily simply connected. The present writers plan to return to this topic on another occasion.

The authors are informed that some results on the asymptotic distribution of zeros of Tchebycheff polynomials have recently been developed also by Dr. J. L. Ullman. The researches contained in the present note have been done independently.

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THE EFFECTS OF CHORIOALLANTOIC TRANSPLANTS OF ADULT CHICKEN TISSUES ON HOMOLOGOUS TISSUES OF THE HOST CHICK EMBRYO

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Weiss has reported that the incorporation of organ fragments (e.g., liver or kidney) from 6-day-old chick embryos into the area vasculosa of 4-day-old chick embryo hosts results in a striking and relatively specific response by the homologous tissue of the host.[1] In the case of liver grafts, for example, after an initial decline, the weights of the hosts' livers steadily increase in comparison with those of controls, until, on the seventh postoperative day, livers of experimental hosts are over 40 per cent heavier than those of controls. In further tests of the organ-specific aspects of the effect, embryonic kidney brei was injected intravenously into younger embryos.[2] Mitotic counts after one or two days showed a 50 per cent increase above normal in the host kidneys, with high electivity of the effect. Weiss has sug-

gested that "either specifically shaped parts of liver cell proteins can act directly as nuclei for further synthesis when entering the appropriate environment, i.e., another liver cell, or they act merely as models for moulds which would turn out more of the original product." He has emphasized that, while the evidence at hand supports the general principle of organ-specific chemical growth regulation by homologous cell constituents, the mode of action is still a matter of conjecture; no selection has been possible between the "building-block" and "template" (catalytic) mechanisms. As a corollary of the theory of organ-specific growth regulation, it is argued that growth and differentiation, being somewhat antagonistic, may react in opposite directions. Hence, under conditions favoring growth, differentiation may be suppressed. Preliminary experiments have been cited in support of this view.[3, 4, 5]

The aim of the present investigation is to test critically the template hypothesis of growth by an immunochemical and biochemical analysis of the specificity, time of origin, and mechanism of action of the factors concerned in the enlargement of host chick embryonic organs following grafts of the homologous tissue from older donors. At the outset of the study the major experimental target was the chick spleen.

It has been established that transplants of adult chicken spleen made to the chorioallantoic membrane of 9-day-old host chick embryos elicit a relatively specific response on the part of the homologous tissue of the host embryo, viz., an increase in mass.[6, 7] The effect is class-specific and quantitatively tissue-specific.[8] Thus, although adult chicken spleen grafts result in a striking increase in weight of the host spleen, grafts of adult mouse spleen are ineffective. Moreover, of eleven adult chicken tissues tested in addition to spleen, only two, thymus and liver, affect the host spleen, and in each case the effect is quantitatively far less than that observed with splenic transplants. It has been shown, in addition, that the ability of chicken spleen grafts to effect host spleen enlargement is correlated directly with the age of the donor, the factor or factors concerned first appearing in the embryonic chick spleen at approximately the fourteenth day of development and increasing thereafter until the adult level is reached. The time of appearance of the specific factors concerned in host spleen enlargement is paralleled by the development of a group of three spleen-specific antigens, as revealed by immunochemical studies. These findings suggest a correlation between the progressive increase with age in the ability of a spleen graft to evoke enlargement of the host's spleen and the differentiation, during a similar period in ontogeny, of a specific group of spleen antigens.[8, 9]

The desirability of extending this line of research is apparent. Among the pertinent questions raised are the following: (1) Is the enlargement of the embryonic chick spleen following a homologous transplant due to an increase in protein, or is it the result of an accretion of smaller molecules? (2) If, as postulated earlier, the enlargement results from a transfer of protein or other tissue-specific molecules, what is the magnitude of the transfer? Is a small number of molecules involved (template or catalytic hypothesis), or is a larger population of molecules transferred? The following experiments were carried out in an attempt to answer these questions. A preliminary report[10] of the findings was made before the American Society of Zoölogists, December 28, 1953.

METHODS AND RESULTS

1. *Determination of Protein Content of Control and Stimulated Embryonic Spleens.* —Is the enlargement of the host embryo spleen following a graft of adult spleen the result of an increased protein content? The following analyses indicate that an affirmative answer to this question is justified. The approach has been to compare the effects on the total and nonprotein nitrogen content of host embryo spleens of the following operative procedures: (1) sham operations, in which the complete chorioallantoic transplantation procedure was carried out but only a drop of sterile Ringer-Locke solution was added; (2) chorioallantoic transplants of adult chicken spleen, inactivated by fixation with cold 95 per cent ethanol, followed by storage at −20° C.; (3) grafts of fresh adult chicken liver; (4) grafts of fresh adult chicken spleen. The chorioallantoic grafting technique employed has been described previously.[7, 8] Donor tissues were from adult fowls of the New Hampshire Red breed, of either sex. Host embryos were from eggs of the same breed of fowls, incubated for 9 days at 37°.5 C. Following the operations, eggs were returned to the incubator for 8 days (total host age at recovery, 17 days), after which the grafts were recovered and fixed in Allen's B-15 or Bouin's picroformol for subsequent histological study. The spleens of the host embryos were removed rapidly to ice-cold 0.15 *M* NaCl, phosphate-buffered at pH 7.0; each spleen was washed carefully in three changes of the saline solution and stored in 1 ml. of buffered saline at −20° C. A total of 308 operations was performed; 249 host spleens were recovered. Of this number, 110 individual spleens were weighed rapidly on a Roller-Smith balance and digested separately in the Kjeldahl reaction mixture recommended by Brüel, Holter, Linderstrøm-Lang, and Rozits.[11] The total nitrogen of each spleen was determined by the micro-Kjeldahl method described by Niederl and Niederl.[12] The results are summarized in Table 1.

TABLE 1

TOTAL NITROGEN OF CONTROL AND ENLARGED SPLEENS

No. Cases	Treatment	Mean Weight of Host Spleens (Mg.) ± Standard Error of Mean	Mean Total Nitrogen (μg/Mg Tissue [Wet]) ± Standard Error of Mean
27	Sham operations	11.2 ± 0.3	16.9 ± 1.0
27	Grafts of inactivated adult chicken spleen	9.7 ± 1.3	17.0 ± 0.8
25	Grafts of fresh adult chicken liver	21.0 ± 1.4	17.5 ± 0.5
31	Grafts of fresh adult chicken spleen	44.4 ± 2.5	16.4 ± 0 5

The remaining 139 spleens were analyzed for both total and nonprotein nitrogen. Spleens were not assayed individually but were pooled in groups of from four to six. Both wet and dry weights of each lot of splenic tissue were determined. Nonprotein nitrogen was determined as the Kjeldahl nitrogen present in an aliquot of a protein-free filtrate of tissue homogenate, the protein being removed by precipitation with trichloroacetic acid. The data are given in Table 2.

Since all the preceding determinations were made on spleens that had been stored at −20° C. for periods ranging from 1 to 17 weeks and thawed before analysis, thus being subject to possible leaching, it seemed advisable to perform a similar set of determinations on fresh material. For this purpose an experiment was set up in

which a total of 84 sham operations and chorioallantoic grafts was made. Eight days later, 73 host spleens were recovered rapidly, as previously described, except that digestions were started immediately. The data clearly substantiate the findings obtained with frozen tissues, namely, that the increase in weight of stimulated spleens is paralleled by an increased nitrogen content.

The mean weight of the host spleens stimulated by homologous grafts is approximately four times that of the controls. The total nitrogen content is similarly increased; on the basis of micrograms of nitrogen per milligram of tissue, there are no significant differences in the several groups. These findings clearly support the view that the increase in weight of host embryo spleens results from an increased protein content.

2. *Selective Incorporation of Radioactivity from Proteins of Labeled Chorioallantoic Grafts of Adult Chicken Tissues into Proteins of Homologous Tissues of Host Chick Embryos.*—Does enlargement of the host spleen result from a transfer of tissue-specific molecules from the transplant to the homologous host tissue? If so, what is the magnitude of the transfer? In attacking this problem, the basic approach involved the transplantation of adult chicken and mouse tissues, labeled with S^{35}, to

TABLE 2

TOTAL AND NONPROTEIN NITROGEN CONTENT OF CONTROL AND ENLARGED HOST SPLEENS

Series	Mean Total Wet Weight (Mg.)	Mean Total Dry Weight (Mg.)	Ratio: Wet Weight/ Dry Weight	Total N (μg/Mg Tissue [Dry])	Nonprotein N (μg/Mg Tissue [Dry])
Sham operated	63.6	11.0	5.8	105	2.9
Inactivated chicken spleen grafts	77.8	13.2	5.8	115	4.3
Fresh chicken liver grafts	102 2	17.3	5.9	106	2.9
Fresh chicken spleen grafts	262.4	43.6	6.0	108	3.2

the chorioallantoic membrane of host embryos. At the time of recovery, the grafts and several host tissues were analyzed to determine the extent of transfer and incorporation of radioactivity.

Adult New Hampshire Red fowls and adult white mice were injected intraperitoneally with S^{35}-labeled L-methionine (Abbott Laboratories). Animals were fasted from 1 to 4 hours prior to, and 12 hours following, the injection, at which time they were sacrificed by decapitation. The standard dosage of labeled methionine injected, 175 μc/kg, was determined in earlier studies, in which a total of 21 adult fowl were injected with a graded series of doses of labeled methionone (21.1–231.4 μc/kg). The objective was to achieve as high a level of activity as possible in the protein fraction of the tissues to be engrafted, without radiation damage. This is accomplished with approximately 175 μc/kg.[13] At sacrifice the following steps were carried out as rapidly as possible: (1) spleen, kidney, liver, gonad, and heart samples were fixed for histologic and radioautographic preparations; (2) spleen, kidney, and liver samples were weighed and stored in the Deepfreeze, pending digestion, for determination of specific activity; (3) the remainder of the spleen and kidney was weighed and used for transplants, which were started within 30 minutes after sacrifice of the donor.

All grafts were made to the chorioallantoic membranes of 9-day-old chick embryo hosts (New Hampshire Red breed). After transplantation, eggs were reincubated at approximately 37°.5 C. for either 3 or 5 days, after which the transplants and the host spleen, liver, and kidney were recovered for analysis. One out of every five grafts, together with its homologous host organ, was fixed in Bouin's picroformol for histologic and radioautographic analysis. All other grafts and the host tissues indicated above were weighed rapidly on a Roller-Smith balance and plunged into ice-cold 0.15 *M* NaCl. Host spleens were pooled in sufficient numbers to weigh at least 50 mg. (wet weight). Host kidneys and livers were pooled in groups corresponding to each group of spleens, and equivalent aliquots were taken after homogenization. All tissues were homogenized and separated into trichloroacetic acid–insoluble and –soluble fractions. Each fraction was then digested to dryness in a mixture of fuming nitric and perchloric acids. The digestate was washed with 6 *N* HCl and again evaporated to dryness. Sulfate was precipitated from an aqueous solution of the digestate by addition of 4 per cent benzidine dihydrochloride in 0.4 *N* HCl and was deposited by filtration on a fritted glass disk. The specific

TABLE 3

INCORPORATION OF RADIOACTIVITY FROM PROTEINS OF LABELED SPLEEN GRAFTS

(Specific activity of host tissue protein/specific activity of graft tissue protein)

	EXPERIMENTAL SERIES			
	1	2	3	4
Host spleen	0.14	0.16	0.04	0.12
Host liver	.04	.07	.018	.05
Host kidney	0.04	0.06	0.016	0.04

TABLE 4

INCORPORATION OF RADIOACTIVITY FROM PROTEINS OF LABELED KIDNEY GRAFTS

(Specific activity of host tissue protein/specific activity of graft tissue protein)

	EXPERIMENTAL SERIES			
	1	2	3	4
Host spleen	0.038	0.06	0.025	0.05
Host liver	.038	.05	.031	.06
Host kidney	0.098	0.13	0.059	0.16

activity of the radiosulfur in benzidine sulfate precipitates of protein and nonprotein fractions of donor and host tissues was determined as counts per minute per micromole of sulfate. The extraction, digestion, and precipitation methods have been described by Forker *et al.*;[14] the filtration method for collection of precipitates has been described fully by Van Slyke *et al.*;[15] hence details of these procedures need not be given here. Radioactivity of the benzidine sulfate precipitates was counted in a methane flow proportional counter.[16] All data are corrected for background, decay, and self-absorption. Sulfate was determined by titration with NaOH, while hot, to the phenol red point.

The following results are based on analysis of donor and host tissues from a total of 902 transplants and injected eggs. The principal object of the study was the spleen. The choice of a second, or control, tissue for comparison lay between heart and kidney, both of which may be transplanted successfully. Adult kidney was selected because its specific activity was considerably higher than that of adult heart. The incorporation into host embryonic tissues of radioactivity from proteins of labeled spleen grafts and labeled kidney grafts, respectively, is shown in

Tables 3 and 4. The data are expressed as specific activity of host tissue protein/specific activity of graft protein; each value is the mean of two samples, determined in duplicate. The results are consistent. They clearly indicate a highly selective transfer of radioactivity from graft to homologous host tissue.

In addition, one series of experiments was performed comparing the incorporation of radioactivity from transplants of labeled chicken and mouse spleen. The donor chickens were injected with 175 μc of labeled methionine/kg of body weight; the donor mice received a somewhat higher dose, 225 μc/kg. The data are summarized in Table 5; each value is the mean of two samples, determined in duplicate. It will be noted that there is no indication of selective incorporation in the chick tissues of radioactivity from transplants of mouse spleen, a fact which corroborates earlier findings relative to the class-specificity of the effect.

TABLE 5

COMPARISON OF INCORPORATION OF RADIOACTIVITY FROM LABELED CHICKEN AND LABELED MOUSE SPLEEN GRAFTS

(Specific activity of host tissue protein/specific activity of graft tissue protein)

Graft	Host Spleen	Host Liver	Host Kidney
Labeled chicken spleen	0.033	0.012	0.013
Labeled mouse spleen	0.009	0.015	0.012

TABLE 6

INCORPORATION OF RADIOACTIVE METHIONINE VIA THE YOLK SAC

SPECIFIC ACTIVITIES OF HOST TISSUES (C.P.M./MICROMOLE OF SULFATE—CORRECTED)

Methionine Injected μc	Treatment before Injection	Spleen	Liver	Kidney
0.045	Sham operation	22.9	17.6	25.4
.045	Transplants nonradioactive spleen	26.6	22.6	24.6
.075	Sham operation	71.3	66.3	57.3
0.075	Transplants nonradioactive spleen	81.5	54.2	106

To summarize the findings thus far: (1) The results show clearly that, following transplantation of adult chicken spleen or kidney, the specific activity of the homologous host tissue is at least 2.4 times that of other embryonic tissues. The transfer is selective both from spleen to spleen and from kidney to kidney. (2) There is no evidence of selective incorporation of radioactivity following transplantation of labeled mouse spleen. (3) In addition, the magnitude of the transfer is greater than might be expected if only a template or catalytic mechanism were involved, for the data indicate that, as early as three days postoperatively, as much as 15 per cent of the host tissue protein may be derived, or contain specific molecular contributions, from the proteins of the graft.

To explore this possibility further, a second major group of experiments was performed. In these experiments, S^{35}-labeled methionine was injected into the yolk sac of 363 nine-day-old embryos immediately following either transplants of nonradioactive spleen or sham operations. Grafts and host tissues were recovered 3 days postoperatively. Although the host spleens were enlarged in the former group, and hence total activity was greater, the specific activities (activity per micromole of sulfate) in the tissues of the two groups did not differ significantly. The data are presented in Table 6. Each value is the mean of two experiments

performed in duplicate. Coupled with the previous findings, these results afford support to the view that the enlargement of embryonic host organs following homologous organ grafts from older donors is due at least in large part to a selective incorporation into the proteins of the host tissue of tissue-specific components from the transplant. Thus the question is raised as to the nature of the specific components transferred. Is it possible that whole cells of the graft are incorporated into the host tissues? Or does the transfer involve subcellular components, e.g., tissue-specific proteins or specific components of the protein molecule larger than amino acids? The data presented in the following section favor the latter view.

3. *Desoxyribose Nucleic Acid Content of Control and Stimulated Embryonic Spleens.*—Microscopic examination of sections of embryonic host kidney from the several control and experimental series offers no support for the first alternative, viz., that whole cells are transferred. The histology of the kidney is sufficiently straightforward so that a comparative study of control and experimental tissues offers little difficulty. Cell counts and measurements of cell size now in progress indicate that the major change in the embryonic host kidney 3 days after the transplantation of adult kidney is an increase in cell size. The mitotic count is increased only slightly. Such data, however, are not obtained readily for host embryo spleens; the spleen is not favorable material for reliable measurements of cell size. In order to obtain quantitative data concerning the possibility of cell transfer in the splenic series, therefore, the following approach has been adopted: It is assumed that if whole (nucleated) cells are transferred, then the desoxyribose nucleic acid (DNA) content of the host spleen should increase concomitantly with the increase in protein. If the transfer is only of protein or specific protein constituents, then the DNA content per milligram of spleen should be lower in the stimulated series than in the control series. It may be argued that this assumption is too great an oversimplification of a complex situation. Growth is, of course, a complex phenomenon which cannot be reduced to the measurement of a single parameter. Moreover, as Weiss has shown, there is an initial burst of mitotic activity in embryonic kidney following homologous grafts. If the splenic response to spleen grafts is comparable, then not only the possibility of transfer of whole cells but an increase in DNA due to stimulation of mitotic activity must be considered. If whole cells or nuclei are not transferred, but mitotic activity is increased, then it is possible that any difference may be obscured, or at least reduced. With these limitations in mind, the DNA content of spleens from the several control and experimental series has been determined. Host spleens are recovered 8 days postoperatively (host age, 17 days) following sham operations, control grafts, or spleen grafts; weighed rapidly; and plunged into ice-cold 0.04 *M* citric acid. Spleens are pooled within the various groups in sufficient numbers to make 100 mg. (wet weight) per determination. Spleens are homogenized, and the DNA extracted by the method described by Schneider.[17] DNA is determined by the diphenylamine reaction (as defined by Dische),[18] and expressed as micrograms DNA per milligram of tissue. Two hundred and forty-five fresh host spleens have been analyzed to date; the data are given in Table 7. These findings also are supported by analyses of 243 additional host spleens which had been stored at $-20°$ C. for varying periods prior to extraction. In both the fresh and the frozen group, it is clear that the DNA content of the enlarged spleens does not increase concomitantly with the increase in

protein, the difference between the DNA contents of control and enlarged spleens being highly significant. These findings are not compatible with the idea that significant numbers of whole cells are transferred from graft to host spleen;

TABLE 7

DNA CONTENT OF ENLARGED AND CONTROL HOST SPLEENS

Series	No. Cases	Mean Wet Weight of Host Spleens (Mg.) ± Standard Error of Mean	DNA of Host Spleens (μg/Mg) ± Standard Error of Mean
Sham operated	73	9.3 ± 0.3	6.7 ± 0.15
Adult chicken heart grafts	99	8.4 ± 0.3	6.5 ± .19
Adult chicken spleen grafts	73	37.1 ± 3.8	4.7 ± 0.21

together with the results discussed previously, they support the idea of a selective incorporation from grafts into homologous host tissues of tissue-specific proteins, or specific constituents of proteins larger than amino acids.

DISCUSSION

The problems of protein biosynthesis and embryogenesis are inextricably interwoven; yet it must be emphasized that the mechanism of protein biosynthesis is only one facet of the more complex question of the forces involved in cellular differentiation and growth. The term "differentiation" encompasses the totality of reactions of which a cell is capable at any given stage and includes the sum total of changes in cell characters from one stage to the next. Contrary to the implications of some definitions,[19] several lines of evidence may be interpreted as indicating that differentiation may occur through the loss or suppression of synthetic activity.[20]

As defined meaningfully by Weiss,[21] growth is the increase in that part of the molecular population of an organic system which is synthesized within that system. Growth is essentially synonymous with reproduction: "The fundamental feature is the capacity of a reproductive system to procreate more systems with similar reproductive faculty." This definition includes only the biosynthesis of proteins and other complex molecules of the self-reproducing type. Implicit in the definition is the fact that growth involves a net increase in protein; whether it results from an increased turnover of protein[22] or an inhibition of protein breakdown,[23] or both, is not clear.

The findings of the present study bear directly both on the specific problem of the mechanism of protein biosynthesis and on the more general question of the interrelationship of protein biosynthesis with differentiation and growth. Let us consider first the mechanism of protein biosynthesis: The processes which convert amino acids into proteins have been studied from several points of view, viz., intracellular site of synthesis, energy requirements, and rate of incorporation. The major experimental targets have been the initial combination of amino acids, the step termed "peptidization" by Lipmann,[24] and the role of possible "template" mechanisms in establishing protein specificity.[25] In addition, the possible role of precursor "type" proteins (urproteins or proteinogens) has been discussed.[26] Until recently, less attention has been paid to reactions of possible intermediates. Bor-

sook *et al.* have studied the composition of several large tissue peptides which may represent stages in protein biosynthesis.[27] Anfinsen and Steinberg have presented data on nonuniform labeling of different residues of the same amino acid in ovalbumin which favor the concept of peptide intermediates.[28] Moreover, from a tracer study of serum albumin synthesis, Peters has concluded that amino acids are in "intermediate compounds" for at least 20 minutes before the fully formed protein can be detected.[29] These intermediates do not resemble the protein in serological properties or electrophoretic or solubility behavior. Snellman and Danielsson have concluded from a study of biosynthesis of reserve globulins in pea seeds that the synthesis is a stepwise phenomenon, involving the formation of oligopeptide intermediates.[30] In addition to the several lines of evidence that favor a stepwise biosynthesis of protein involving peptide intermediates, one recent investigation has produced evidence which strongly suggests that embryonic cells may utilize a protein nutrient source without the release of free amino acids. Francis and Winnick have compared the mode of utilization by tissue cultures of cardiac muscle of free C^{14}-labeled amino acids with that of the same amino acids in protein-bound form.[31] From experiments in which relatively large concentrations of nonisotopic amino acids were added to the medium as metabolic traps, it was concluded that most of the C^{14} singly labeled proteins could be transferred from a nutrient embryo extract medium to heart muscle protein without the release of free amino acids. A similar conclusion was reached in experiments with doubly labeled proteins. Despite the convergence of opinion indicated in the several lines of investigation cited above, the facts available are not consistent enough to warrant any generalization. Muir, Neuberger, and Perrone, for instance, have presented data which indicate that there are no differences in specific activities of valine at terminal and nonterminal positions of hemoglobin synthesized by the rat supplied with the labeled amino acid.[32] Halvorson and Spiegelman have concluded that already formed proteins have no direct role in the formation of adaptive enzymes without passing through the free amino acid pool.[33]

The findings of the present investigation offer strong support for the conclusion of Francis and Winnick that embryonic cells can utilize tissue proteins without first breaking down the proteins into free amino acids, and, in fact, they favor the further conclusion that the incorporation is of a highly specific nature, e.g., that splenic proteins or specific constituents thereof are selectively incorporated by the developing spleen, a possibility which was not tested in the experiments by Francis and Winnick. It appears unlikely that the degree of electivity reported here could be accounted for on the basis of transfer of amino acids. The conclusion that the specific effect of grafts upon homologous host tissues is due to a selective incorporation from grafts into proteins of the host tissues of tissue-specific protein constituents larger than amino acids must be regarded as tentative, pending the outcome of further experiments. A consideration of the data, together with the findings of others, however, indicates this view to be acceptable as a working hypothesis, subject to further test.

It will be of interest to examine next the bearing of this working hypothesis on the concept of organ-specific growth control. The general principle of the control of of growth by organ-specific substances has been subjected to several experimental tests. The establishment of the class-specific and quantitative organ-specific as-

pects of the principle, and the conclusion that the increase in size of homologous tissues elicited by grafts results from a true increase in protein content, are based on a consideration of the data obtained in a sustained investigation involving, to date, analyses of graft and host tissues of 3,446 chorioallantoic transplants and variously injected and sham-operated embryos, employing the techniques of bio-, immuno-, and radiochemistry in conjunction with the more classical methods of experimental embryology. With respect to the mechanism of action of organ-specific growth-controlling substances, the working hypothesis suggested above permits us to focus on several clear experimental targets. It should be possible, for example, to determine the postoperative time required for substances from the graft to reach the homologous organ of the host, together with the subsequent rate of transfer. These data, correlated with the increase in mitotic rate and rate of increase in weight of the host organ, should permit a critical evaluation of the "building-block" hypothesis suggested here, as compared with a possible template or catalytic mechanism. The findings in the investigation thus far strongly suggest that a "building-block" mechanism is responsible, but they do not rule out completely the involvement of a catalytic mechanism. The hypotheses are not mutually exclusive. A second question of interest concerns the intracellular site of localization of the transferred molecules, particularly as related to present concepts of the site of protein synthesis in the cytoplasm.[34] For example, does the localization of graft substances parallel the intracellular localization of antigens in adult organisms?[35]

In conclusion, let us consider the bearing of the present study on the related problem of the inhibition of tissue differentiation by fragments, extracts, or granular fractions of homologous older organs. It has been reported that extracts of embryonic chick heart inhibit differentiation (pulsation) of growing cardiac tissue in vitro[3] and that the differentiation of embryonic frog tissues (e.g., blood, brain, heart) is suppressed by culturing embryos through the neurula stage in a culture medium containing suitable concentrations of products of the homologous adult tissues.[4] More recently, Shaver has reported that a granular fraction of frog brain arrests brain differentiation.[5] Unfortunately, it is not possible to evaluate these reports critically, since the data have not yet been published. It can be argued on theoretical grounds that the two lines of evidence involving stimulation of growth and suppression of differentiation are not in conflict. It is clear in the case of both spleen and kidney grafts studied in this laboratory that the pattern of histogenesis of the host organs is unaltered. One might ask, however, whether a splenic graft made to the chick embryo early in the fourth day of development, well before the spleen has made its appearance, might affect differentiation rather than growth. All too frequently, such apparent conflicts are written off as being due simply to the antagonistic nature of growth and differentiation, a most uninstructive conclusion. A detailed study of the differences in methodology, conditions of host or affected embryos (normal, aged, or overripe, etc.) and state of development of target tissues may provide a basis for interpreting the present differences in results and, in addition, afford another point of departure in our analysis of the mechanisms of growth and differentiation.

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