THE EFFECTS OF ADULT TISSUE FRACTIONS AND OF ANTIBODIES AGAINST THESE FRACTIONS ON THE EARLY DEVELOPMENT OF RANA PIPIENS EMBRYOS

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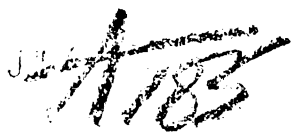

ABSTRACT

THE EFFECTS OF ADULT TISSUE FRACTIONS AND OF ANTIBODIES AGAINST THESE FRACTIONS ON THE EARLY DEVELOPMENT OF RANA PIPIENS EMBRYOS

By

Pamela Kay Mc Allister

Three experiments were conducted in an attempt to clarify the role of both antigens and antisera in the processes of early growth and differentiation in Rana pipiens embryos.

Hearts and kidneys from adult frogs were fractionated and the fractions and antibodies against them were injected into embryos in the blastula stage.

Tissue fractions from both heart and kidney caused inhibition of development in a significant number of embryos. The effects were non-specific in that the development of the entire embryo was retarded.

The injection of absorbed and unabsorbed antisera against heart fractions inhibited the development of the heart in a small number of embryos. The results were specific in that other organs developed normally. Those embryos injected with anti-kidney or with control sera exibited no defects of the heart.

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INTRODUCTION

It has been claimed in recent years that the application to an embryo of organ or tissue specific antigens or of antibodies directed against those antigens could produce either a stimulatory or inhibitory effect on the differentiation of the homologous organ or tissue depending on the experimental conditions. Growth, cell interactions, cell migration, and differentiation may all be affected by both antigen and antibody and the net effect may be either positive or negative. The effects of the antigens or antisera may be species-specific or tissue-specific or both, or may be totally non-specific. Specificity of the stage of development at which the antigens and antibodies exert their effect has also been noted.

Numerous experiments have been done to determine the effect of adult tissues both in developing and in regenerating systems. Weiss (1952) proposed that adult tissues have an inhibitory effect on the homologous organ in a developing embryo. He cultured chick embryos in extracts with or without the homologous organ. Fewer of those embryos cultured in whole embryo extract containing the homologous organ developed hearts or kidneys than those cultured in incomplete extracts lacking either heart or kidney.

Rose (1955) demonstrated that in some cases the presence of adult tissues could specifically inhibit the development of the homologous organ in Rana pipiens embryos. In most cases the embryos were retarded at a specific stage of growth. Specific effects were limited to 4 of

26 experiments. Brain-treated animals in these 4 experiments exibited poor morphology of the nervous system, incomplete or missing neural canal, and random arrangement of cells in the neural tube. Heart-treated embryos had small hearts, and those treated with blood failed to develop blood cells.

Clark and McCallion (1959) demonstrated that the effects of organ homogenates were specific since heart homogenates cause reduction in heart size but have no effect on the brain. More recently, McCallion (1964) working with chick embryos has demonstrated that the inhibitory effect of brain on the development of the brain is related to organ specific substances. Brain specific proteins when injected between 32 and 96 hours result in abnormal growth of the nervous system. Injection of brain proteins and lipoproteins which are also present in other tissues have no such effect on the nervous system.

Braverman (1961) demonstrated that the effect of the injection of brain is not only specifically inhibitory to the brain but is specific for that region of the brain from which the extracts are derived. Extracts from the anterior portion of the brain preferentially inhibit the differentiation of the forebrain, whereas extracts from the hindbrain inhibit the hindbrain and all areas more anterior. Thus there may be a gradient effect such as that demonstrated in regenerating systems.

That adult organs have a stimulatory effect on the homologous organ has also been suggested and is supported principally by experiments employing chorioallantoic grafts in the chick. Ebert (1951) observed spleen hypertrophy following adult spleen grafts. Ebert (1954) further found that the nitrogen content of the host spleen increased indicating an increase of protein content of the organ. He also demonstrated that the increased nitrogen content of the host spleen resulted from

mobilization of S^{35}- methionine labeled proteins from the graft.

Tumanishvili and Salmatina (1968) found that embryonic chick liver increases in size upon the injection into chick embryos of adult liver cytoplasm. The increase in liver size followed a burst of mitotic activity. They further demonstrated that injection of nuclear material results in inhibition of mitotic activity and a decrease in liver size. Liver size in these experiments was determined by two factors within the cytoplasm and nucleus which stimulate and inhibit mitosis.

That the effect of some adult tissues is non-specific was demonstrated by Van Alten and Fennell (1959). Embryonic chick spleen was enlarged following grafts of many organs. Both liver and heart were enlarged following spleen, liver, and duodenal grafts.

Andres (1955) measured mitotic indices of liver and mesonephros following the injection of a suspension of liver or mesonephros into chick embryos. The mitotic index of the homologous organ increased while that of the heterologous organ remained the same, was slightly decreased, or was increased but to a lesser extent than the homologous organ.

There are several factors which must be taken into account when considering the effect of adult tissues on developing systems which may account for the contradictory results which have been obtained in the works previously described. The spleen is an immunologically competent organ and thus the increase in size of the host spleen demonstrated by Ebert, and of other organs demonstrated by Van Alten and Fennell, may be a result of a graft-versus-host reaction. Such spleen enlargement has been demonstrated by Simonsen (1962) who used genetic markers to determine that spleen enlargement in chick embryos was due to the invasion of immunocompetent cells into the host spleen.

Further, the mode of response to adult tissues may be dependent on time. Undifferentiated tissues appear to be subject to inhibitory control whereas differentiated tissues which have not yet attained adult size may not.

Although the effects of tissue antigens on development may be unpredictable, the effects of antisera do not appear to be so. Antisera may be lethal if applied in high doses or may produce many non-specific defects. Antisera prepared against body tissues may also produce specific defects if applied directly to the embryo in the proper concentration.

Lens antiserum, applied in a suitable concentration, has been effective in interfering with lens and eye development (Fowler and Clarke, 1960; Clarke and Fowler, 1960; Langman, 1960, 1963). The reaction of the embryos was limited to ectodermal tissues and was effective only if the antisera were applied before the 16 somite stage (Langman, 1960). Langman (1963) also demonstrated that the antibody responsible was directed against the α-crystalline.

Ebert (1950) studied the effect of tissue antisera on the developing chick. The antisera were lethal in high concentrations and inhibited growth in lower concentrations. At very low concentrations the antisera produced specific defects in the developing embryos. Those embryos grown in anti-heart sera frequently failed to develop normal somites and lateral plate mesoderm and occasionally failed to develop functional hearts. Neural development in these embryos was normal. Those embryos cultured in anti-brain sera had defects in the anterior portion of the brain.

Owens (1960) injected early frog blastulae with anti-heart sera. Heart development was selectively inhibited in a significant number of embryos. The inhibition could not be coorelated with the presence or

absence of heart-specific antibodies in the sera.

The effects of antisera in mammalian development have also been extensively explored. A review of this work can be found in Brent (1968).

If antisera have an inhibitory effect on developing organs then it could be predicted that antigens, being complementary, would have the opposite effect and would thus stimulate the development of the homologous organ. This study was begun in an attempt to clarify the role of both antigens and antisera in the processes of early growth and differentiation in Rana pipiens. It was hoped that the use of substances of greater tissue specificity than employed in past experiments would result in more predictable results, and that the effects of both the antigens and antisera could be related to the specificity of the substances used.

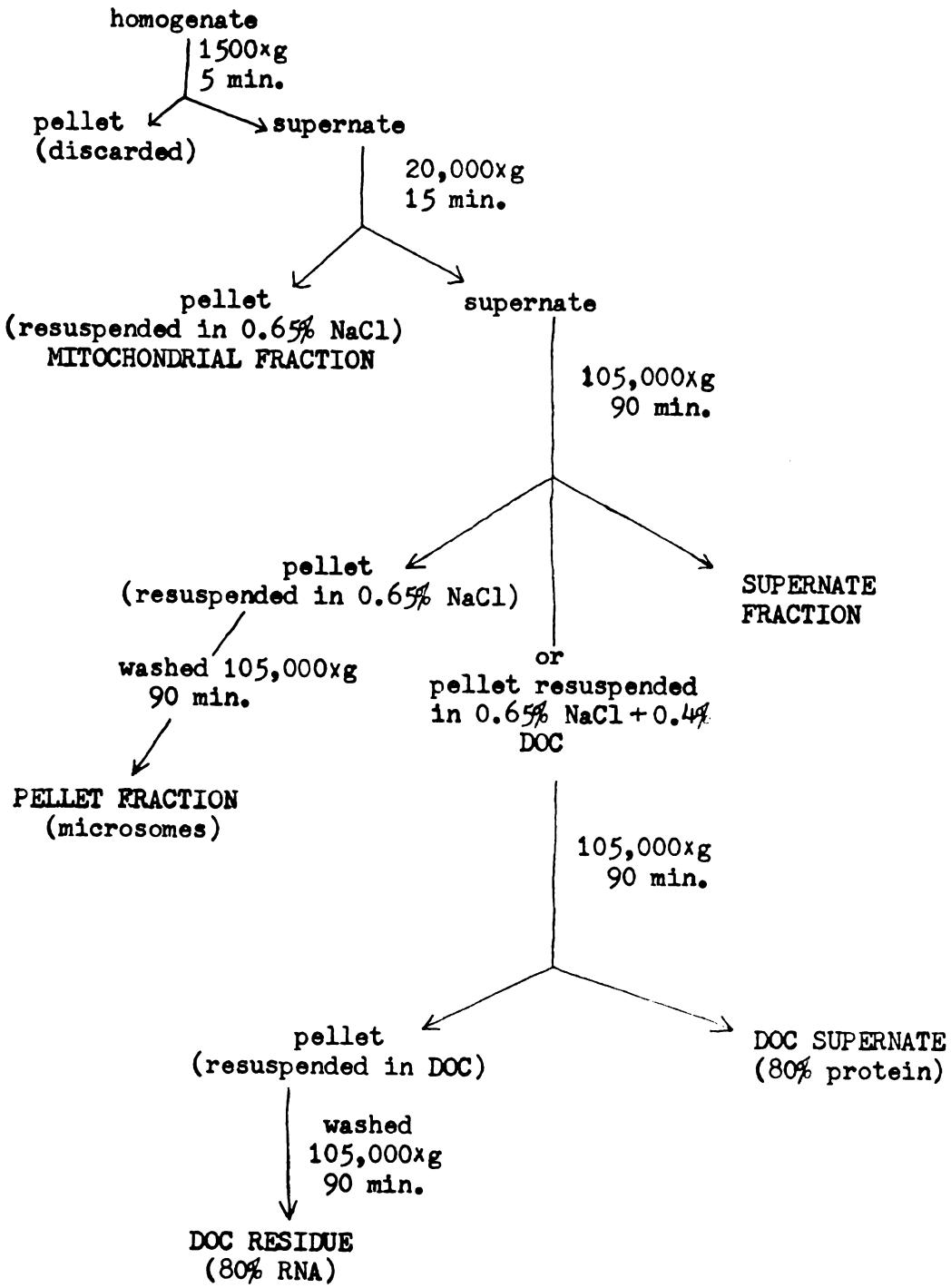
METHODS AND MATERIALS

A. Preparation of Antigens:

Antigens were prepared by a modification of the method of D'Amelio and Perlman (1960). Thirty to thirty-six large frogs are pithed and the heads removed. The hearts and kidneys are removed, washed, blotted on filter paper, and weighed. The tissues are then ground in a glass grinder using 1 gram of tissue to 10 ml. of medium. The kidneys are ground in 0.24 M sucrose in 0.02 M phosphate buffer at a pH of 7.2. The hearts are ground in 0.24 M urea in phosphate buffer. The homogenate is then centrifuged according to the scheme in Figure 1. The fractions produced are used for injection into rabbits to produce antibodies and also for injection into embryos.

B. Preparation of antisera:

All antigens were injected into adult rabbits (German checker strain) in the subscapular musculature using 1.5 ml. of antigen solution and 1.5 ml. of complete Freund's adjuvant. The rabbits were given booster injections at one month intervals following the initial injection. Incomplete Freund's adjuvant was used for the booster injections. The rabbits were bled through the marginal ear vein one week after each booster injection. The antisera were tested in Ouchterlony double diffusion plates using 1% ion agar number 2 (Colab) in rabbit saline. The antisera were tested against the homologous antigen solution and against various heterologous antigen fractions. The antisera were then



Centrifugation Procedure for Tissue Fractionation

Figure 1

pooled and fractionated with 33% ammonium sulfate to separate the globulin and albumen fractions. The globulins were dialized against 0.6% NaCl, frozen, and stored until use.

C. Injection of Embryos:

Adult female frogs were stimulated to ovulate according to the procedure of Wright and Flathers (1961). Twenty-four to forty-eight hours later the eggs were squeezed onto glass slides and fertilized. Sperm suspension was obtained by macerating the testes from two adult males in 0.1 strength Holtfreter's solution. Seven to ten ml. of Holtfreter's was used for each testis. After fertilization the eggs were placed in aerated tap water in finger bowls and kept at 18 degrees until they reached the mid-blastula stage at which time they were injected. Injections were performed with glass micropipettes prepared from capillary tubing (1.0 mm O. D.) with a Livingston pipette puller (Otto K. Hebel,, Scientific Instruments, Rutledge, Pa.). A 10 mm. hypodermic syringe was connected to the injecting needle by a length of Intramedic polyethylene tubing (I.D. 0.030" & O.D. 0.048", Clay Adams, Inc., N.Y.) and the system was then filled with water. The needle was filled by withdrawing the plunger of the syringe leaving an air bubble between the water and the injecting fluid. Injections were performed under a binocular dissecting microscope by inserting the needle into the blastocoel cavity and expelling the fluid by squeezing the plunger. Embryos from each female were injected with both control and experimental solutions. Holtfreter's solution was used as a control in all experiments. In those experiments in which antibodies were used, non-immune serum was also used as a control. Non-immune serum absorbed with liver was used as an additional control

in the experiment utilizing absorbed antisera.

D. Histological Preparation:

After injection, the embryos were maintained at 18 degrees until the controls reached Shumway stage 22 at which time they were fixed in Smith's modification of Bouin's fixative. Randomly selected embryos from each group were then embedded in paraffin, sectioned at 10 microns, stained in hematoxylin and eosin, and examined histologically.

RESULTS

A. Analysis of antigens and antisera:

The analysis of the heart tissue fractions showed that each fraction had antigenic determinants in common with the other fractions and in common with kidney (Figure 2). The mitochondrial fraction had fewer antigenically active components than the supernate or the pellet. The mitochondrial antigens were not unique to the mitochondrial fraction since they were also present in the supernate and in the pellet. Heart supernate and whole heart apparently contain the same number and kinds of antigenic determinants when tested with unabsorbed antisera. The uncertainty is due to the large number of precipitin lines formed. The heart pellet produced fewer precipitin lines, none of which were unique to the pellet. Using antisera absorbed with liver, it was readily apparent that the supernate either with sucrose or with urea buffer contained at least one antigenic determinant not found in any other fraction. Absorption with other body tissues also failed to remove this component. After absorption with liver, 3 precipitin lines remained. Two of these could be removed by absorption with frog serum, kidney, or skeletal muscle. The third line remained after absorption with these tissues.

The analysis of the kidney tissue fractions showed that all the fractions had many determinants in common with heart (Figure 3, a-e). The mitochondrial fraction, supernate, and pellet appeared to be immunologically alike and were indistinguishable from the whole kidney

Figure 2--Analysis of heart fractions.

Key to Abbreviations

AH - Anti-whole heart serum
AK - Anti-whole kidney serum
HS - heart supernate
HUS - heart urea supernate
H - whole heart
HM - heart mitochondria
HP - heart pellet
H DOC S - heart DOC supernate
H DOC R - heart DOC residue
K - whole kidney

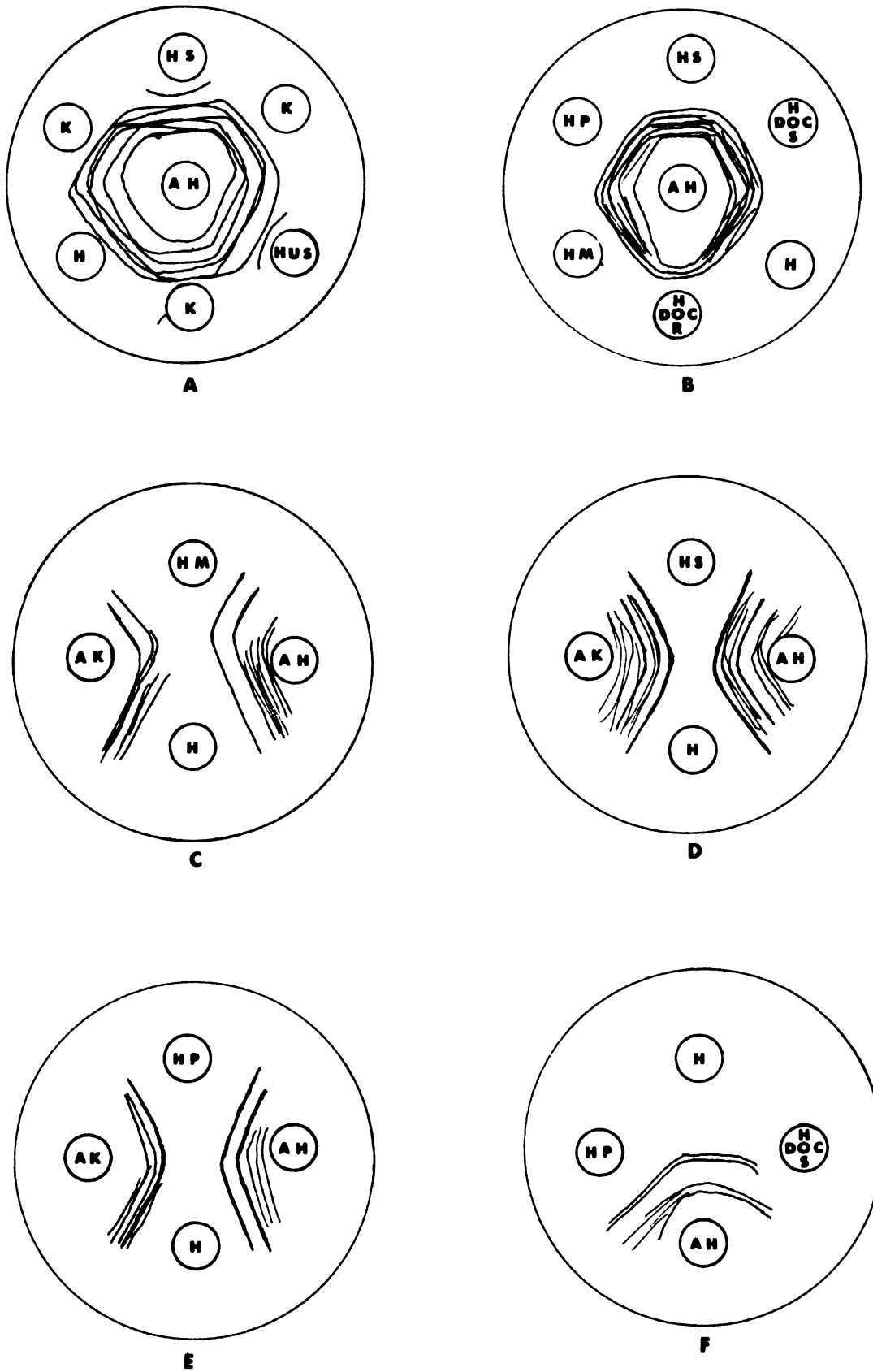

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Figure 2

Figure 3--Analysis of kidney fractions.

Key to Abbreviations

AK - Anti-whole kidney
AH - Anti-whole heart
K - whole kidney
KM - kidney mitochondria
KS - kidney supernate
KP - kidney pellet
K DOC R - kidney DOC residue
K DOC S - kidney DOC supernate
H - whole heart
AKL - Anti-kidney absorbed with liver

homogenate. The DOC residue was only weakly antigenic and produced few precipitin lines when tested against anti-whole kidney. The DOC supernate did not differ from the pellet. When antisera were absorbed with liver, all fractions except the DOC residue formed one or more precipitin lines with the anti-whole kidney sera.

The unabsorbed antisera which were used for injection into embryos contained many antibodies which were not specific for the tissue against which they were directed. That is, all antisera contained antibodies against antigenic determinants found in other body tissues as well. In all cases except the anti-heart pellet the antisera also contained organ-specific antibodies. Antisera after absorption in most cases no longer reacted with heterologous tissues. Anti-heart supernate and anti-heart urea supernate formed two weak precipitin lines with kidney after absorption (Figure 4, c-e). Anti-heart pellet antisera after absorption no longer formed precipitin lines with either heart or kidney (Figure 4, f). Anti-kidney sera after absorption no longer reacted with heart but would react with heterologous kidney fractions (Figure 3, f; Figure 4, a&b).

B. Effect of antigen injection:

The embryos injected with tissue fractions frequently failed to develop beyond the tailbud stage (Figure 5). All of the heart fractions injected produced this effect in a significant number of cases (Table 1). Those embryos injected with undiluted heart supernate died shortly after gastrulation. Those embryos injected with whole kidney or kidney supernate also failed to develop beyond the early tailbud stage. The kidney supernate was lethal to 90% of the embryos injected with it. The supernate diluted 1:10 and the kidney pellet did not produce significant numbers of retarded embryos. No embryos were found in which the

Figure 4--Analysis of antisera

Key to Abbreviations

AKSL - Anti-kidney supernate absorbed with liver
AKPL - Anti-kidney pellet absorbed with liver
AHL - Anti-whole heart absorbed with liver
AHSL - Anti-heart supernate absorbed with liver
AHUSL - Anti-heart urea supernate absorbed with liver
AHPL - Anti-heart pellet absorbed with liver
K - whole kidney
KM - kidney mitochondria
KS - kidney supernate
KP - kidney pellet
H - whole heart
HM - heart mitochondria
HS - heart supernate
HUS - heart urea supernate
HP - heart pellet
H DOC S - heart DOC supernate

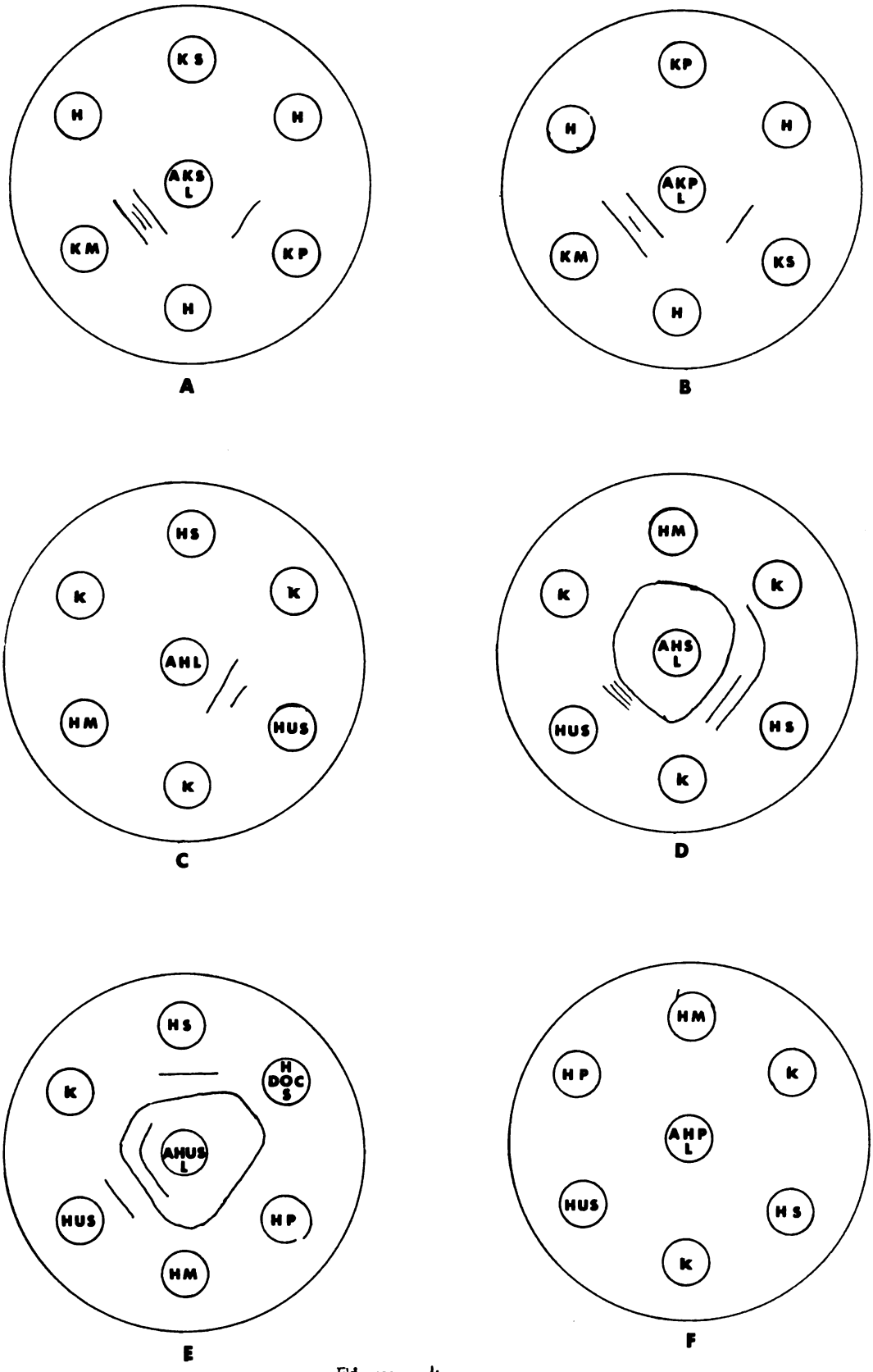
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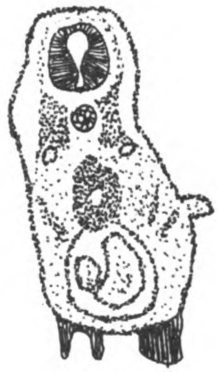
Figure 4

Figure 5--Embryos injected with tissue fractions.

A - Control embryo injected with Holtfreter's solution. Section through the heart.
B - Same embryo as above. Section through the pronephros.
C - Embryo injected with heart supernate diluted 1:10. Section through the heart.
D - Same embryo as above. Section through the pronephros.
E - Embryo injected with kidney supernate. Section through the heart.
F - Same embryo as above. Section through the pronephros.



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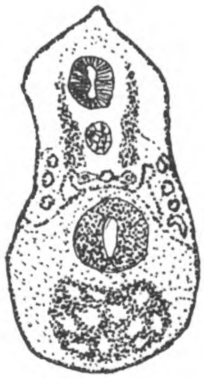

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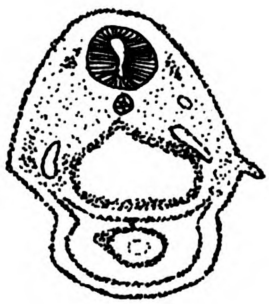

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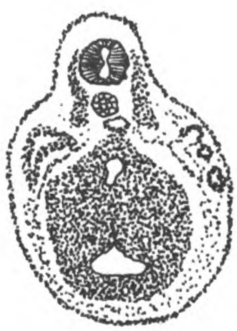

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Figure 5

Table 1--Results of Tissue Fraction Injection

INJECTED WITH		DEVELOPMENT RETARDED	NORMAL	PROBABILITY (BINOMIAL)
Control	Holtfreter's	5	25	--
Heart	whole[a]	3	3	0.06
	supernate[a]	(lethal)	-	--
	supernate 1:10[a]	4	2	0.008
	urea supernate[a]	3	3	0.06
	pellet[b]	3	3	0.06
Kidney	whole[a]	2	4	>0.25
	supernate[a]	(90% lethal) 4	2	0.008
	supernate 1:10[a]	1	5	>0.50
	pellet[a]	1	5	>0.50

a - tissue-specific antigen demonstrated
b - tissue-specific antigen not demonstrated

heart or kidney was specifically affected.

C. Effects of antisera injection.

The injection of unabsorbed antisera did not cause cessation of development as was observed in the antigen injected embryos. The injection of anti-heart sera produced embryos with specifically reduced hearts in a few instances (Figure 6, Table 2). These embryos were otherwise normal. No control embryos were found with specifically reduced hearts. No specific inhibition of kidney development were observed.

Antisera absorbed with liver produced embryos whose development was retarded at the tailbud stage in many instances. This was seen in those embryos injected with absorbed control serum as well as those injected with absorbed tissue antisera.

In addition, specific inhibition of heart development resulted from the injection of antisera against heart fractions (Figure 7, Table 3). The injection of kidney antisera had no specific effect on development which could be determined. Specific inhibition was found both in antisera in which tissue-specific antibodies were present and in sera in which no specificity could be demonstrated. Further, antisera in which a specific component was demonstrated failed in some cases to produce inhibition of the homologous organ. In no case was inhibition of development of a heterologous organ observed.

Figure 6--Embryos injected with unabsorbed antisera.

A - Control embryo injected with non-immune serum. Section through the heart.
B - Same embryo as above. Section through the pronephros.
C - Embryo injected with anti-heart pellet. Section through the heart.
D - Same embryo as above. Section through the pronephros.
E - Embryo injected with anti-kidney supernate. Section through the pronephros.
F - Same embryo as above. Section through the pronephros.

A

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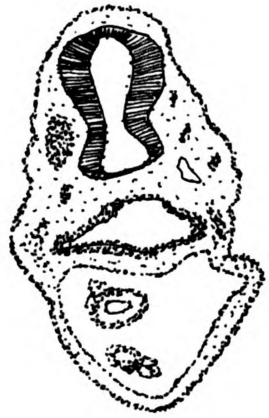

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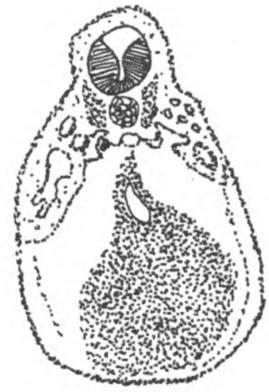

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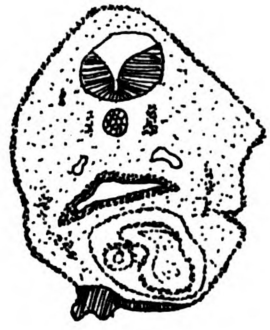

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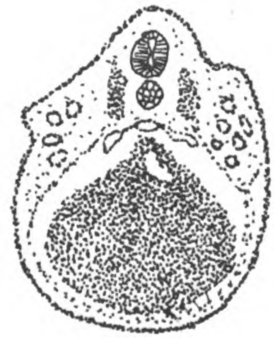

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Figure 6

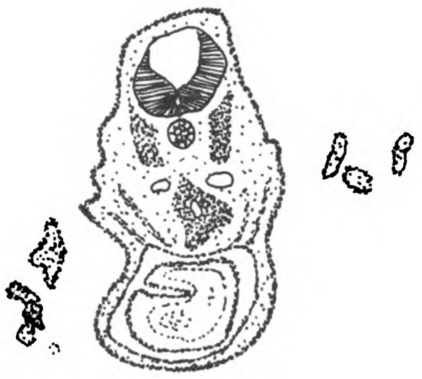
Table 2--Results of Unabsorbed Antiserum Injection

INJECTED WITH		DEVELOPMENT RETARDED	NORMAL	HEART REDUCED	KIDNEY REDUCED
Control	Holtfreter's	0	13	0	0
	non-immune serum	2	13	0	0
Anti-Heart	supernate[a]	0	5	2	0
	urea supernate[a]	0	11	2	0
	pellet[b]	0	4	2	0
Anti-Kidney	supernate[a]	0	7	0	0
	pellet[a]	2	7	0	0

a - organ-specific antibodies demonstrated
b - organ-specific antibodies not demonstrated

Figure 7--Embryos injected with absorbed antisera.

A - Embryo injected with non-immune serum absorbed with liver. Section through the heart.
B - Same embryo as above. Section through the pronephros.
C - Embryo injected with anti-heart pellet absorbed with liver. Section through the heart.
D - Same embryo as above. Section through the pronephros.
E - Embryo injected with anti-kidney pellet absorbed with liver. Section through the heart.
F - Same embryo as above. Section through the pronephros.



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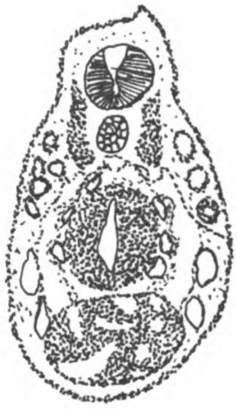

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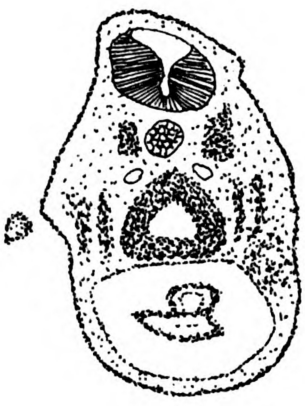

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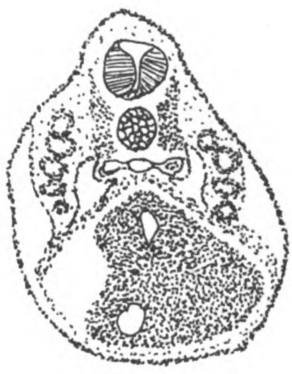

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Figure 7

Table 3--Results of Absorbed Antiserum Injection

INJECTED WITH		NORMAL	DEVELOPMENT RETARDED	HEART REDUCED	KIDNEY REDUCED
Control	Holtfreter's	5	1	0	0
	non-immune serum	6	0	0	0
	absorbed non-immune serum	7	5	0	0
Absorbed Anti-Heart	whole[a]	3	1	2	0
	supernate[a]	3	2	1	0
	urea supernate[a]	0	6	0	0
	pellet[b]	5	0	1	0
Absorbed Anti-Kidney	whole[a]	6	0	0	0
	supernate[a]	3	3	0	0
	pellet[a]	6	0	0	0

a - organ-specific antibodies demonstrated
b - organ-specific antibodies not demonstrated

DISCUSSION

As was discussed in the introduction it was hoped that by fractionation of the adult organs, more highly specific antisera could be produced. The fractionation procedure employed here failed to produce separation of distinct antigenic components. The differences between the fractions were in most cases of a quantitative nature only. Fractionation of the kidney was unsuccessful in that all fractions were indistinguishable upon analysis and organ-specific components could be demonstrated in all except the DOC residue.

Heart fractionation proved slightly more successful since a heart specific antigen or antigens were limited to the supernate fraction. The supernate in addition contained many non-specific antigens.

Highly specific anti-organ sera could not be produced by the injection of the above mentioned tissue fractions since they are all highly non-specific in nature. Absorption of the antisera with liver removed most of the non-specific antibodies leaving the sera highly organ specific. Liver antigens, however, were not entirely removed after the absorptions so that the antisera which were injected into the embryos contained liver protein in addition to antibodies.

That tissue homogenates may inhibit the development of the homologous organ has been suggested by many experiments. The specificity of these effects however has not been demonstrated. Frequently, organs were scored as inhibited if they were in any way abnormal when in fact the abnormality could have been due to hypertrophic growth (Rose, 1955;

McCallion, 1964; Braverman, 1961). The presence of a solid nervous system could result from proliferation of additional nervous tissue as well as from an inhibitory effect on differentiation producing an abnormal nervous system.

Evidence for organ specific stimulation is derived principally from studies using chorioallantoic grafts of the spleen or experiments utilizing injection of tissue homogenates. These effects are not specific in that other organs also exibited increases in size or mitotic index.

The experiments described in this paper indicate that the effect of tissue fractions is non-specific since the development of the entire embryo is retarded.

Rose (1955) also found that heart tissue would inhibit development beyond the early tailbud stage. Upon dilution he noted that the tissue homogenate would specifically inhibit heart development. Dilution of the tissue fraction had no such effect in the experiment described in this paper, but rather resulted in the production of fewer retarded embryos. It is possible that a critical concentration was never reached since the protein concentration of the fractions was not measured and dilutions were performed in only two cases due to the lethal effect of the undiluted fractions.

The inhibitory effect of the tissue homogenates may be due to a stimulation of mitosis since a mitosis stimulating protein has been found in the cytoplasm of liver and is presumably present in all tissues (Tumanishvili and Salmatina, 1968). Stimulation of the mitotic rate would prevent differentiation since these activities are mutually antagonistic (Weiss, 1955). Thus inhibition of development may be an

indirect effect of increased mitotic activity.

It is also possible that enzymes present in the tissue fractions may interfere with normal metabolic processes in the embryos and thus prevent them from developing beyond the tailbud stage.

Analysis of the antisera indicated that little difference exists between the antisera produced against different fractions of an organ or between organs. However, organ specific components could be demonstrated in most of the sera.

When unabsorbed antisera were injected into the embryos their development was slightly delayed in some cases, but the inhibition was never as severe as when tissue antigens were injected. Other types of abnormalities were also seen in the embryos injected with antisera. Many embryos were edematous and had curvature of the spine. These effects are probably due to a general teratological effect of the sera since they occurred in all groups injected with serum and were not coorelated with specific defects. In a small number of embryos treated with anti-heart sera specific reduction of heart size was seen. Reduction of heart size was not seen in control or in kidney injected embryos. The specific reduction in heart size could not be coorelated with the presence of heart-specific antibodies, since embryos treated with anti-heart pellet serum had reduced hearts although no heart-specific antibodies could be demonstrated in this serum. It is probable, however, that specific antibodies were present but were not detected by the method used.

Unabsorbed kidney antisera did not produce inhibition of pronephric development. There were many difficulties encountered when trying to determine the effects of the antisera on the pronephros. Edema in many of the embryos resulted in distortion of the pronephros so that the Wolffian ducts and tubules were greatly enlarged and had very thin walls.

The enlargement was seen only in edematous embryos and was presumably due to the edema. Further, the number and size of the tubules were highly variable even in control embryos so that it was not possible to determine if the pronephros was smaller in the antiserum-treated embryos. Two possibilities exist; either the inhibition of development was present but was not detected, or no inhibition was produced by the sera. The antibodies were directed against the adult mesonephros which may be antigenically different from the pronephros and these antibodies may then be unable to affect the developing pronephros.

Of those embryos treated with absorbed antisera many failed to develop beyond the tailbud stage. This effect is most likely due to the presence of liver in the injecting antisera, since this effect was also seen in the embryos injected with tissue fractions. That is, liver may contain the same type of a general inhibitory substance as tissue fractions. The effect was not seen in those embryos treated with unabsorbed antisera.

As was the case with unabsorbed antisera, small numbers of embryos were found in which heart development was suppressed after treatment with absorbed antisera although other organs were normal. The defects were not limited to those sera in which organ-specific antibodies were demonstrated.

It cannot be determined from the experiments described here how the antibodies are affecting the embryos although several theories have been proposed which may be pertinent.

Weiss (1953) suggested that the development of an organ is determined by the presence of complemantary substances. A cellular template directs the synthesis of additional templates resulting in organ growth. Diffusible anti-templates are then produced and when the concentration of these anti-templates reaches a critical level development ceases.

This theory is based on experiments in which the mitotic indices of liver and mesonephros increased after an initial decline when treated with the homologous organ. According to Weiss' theory, organ-specific substances (templates) stimulate the growth of the homologous organ. Such stimulation has been described in cases in which the organ was differentiated but had not yet attained adult size, but has never been demonstrated in a differentiating system. Conceptually, templates and anti-templates may play a role in determining the ultimate size that an organ may reach, but appear to have no role in controlling the primary differentiation of an organ. Further, the presence of a mitotic stimulator and of a mitotic inhibitor in the cytoplasm and nuclei of cells respectively has been demonstrated by Tumanishvili and Salmatina (1968). They have demonstrated that it is the relative concentrations of these two substances which control the mitotic index and thus the size of embryonic liver. Thus the results described here might as well be due to such a stimulation-inhibition balance as to a template-anti-template conception.

The specific inhibition theory of Rose (1955), contrary to that of Weiss, suggests that substances from adult tissues are inhibitory to homologous differentiating tissues. One would expect on the basis of this theory that antibodies, being complementary to the adult tissues would inactivate the inhibitory factor produced by the cells as they differentiated and as a result would cause hypertrophic growth. However, hypertrophic growth as envisaged by this theory has never been demonstrated.

According to the building block theory of Ebert (1951, 1954), cells may incorporate preformed proteins or may utilize these proteins in the synthesis of new proteins. Thus, grafts of adult tissues would cause hypertrophic growth due to the mobilization of preformed substances

which may be present in the yolk. The present results could be interpreted in terms of an interference with the synthetic processes preceeding specific differentiations.

It is also possible that the antisera may be inactivating specific inductive processes necessary for cellular differentiation.

SUMMARY

Since highly organ specific antisera were not obtained by the procedures described in this paper it was not possible to determine if the effects of the injection of antigens and antibodies into embryos were coorelated with the presence of organ-specific substances.

The injection of tissue homogenates produced no specific inhibition, but rather resulted in general inhibition of development. Injection of anti-heart sera resulted in the production of a small number of embryos with hearts which were either very small or absent altogether, whereas injection of anti-kidney sera had no detectible effect on the embryos. Injection of antisera absorbed with liver had the same effect as injecting unabsorbed antisera and in addition inhibited development of the entire embryo presumably because of residual liver protein present in the sera.

The experiments described here give no information either as to the site of localization of the injected substances, if any, or as to the time at which these substances act. Many possibilities as to the mode of action of the antigens and antibodies has been discussed but it is not possible to determine on the basis of these experiments which, if any, of these may have produced the results described.

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