THE EFFECT OF CHORIOALLANTOIC GRAFTS ON THE DEVELOPING CHICK EMBRYO WITH SPECIAL REFERENCE TO THE DEVELOPMENT OF ANTIGENS IN THE DUODENUM

By

Pierson J. Van Alten

AN ABSTRACT

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THESIS ABSTRACT

Various tissues from adult chickens (duodenum, liver, spleen, heart, brain and skin) and embryonic duodena (20-, 18-, 17-, 16-, and 15-day) were grafted to the chorio-allantoic membrane (CAM) of 9-day chick embryos and the whole chick, spleen, liver, heart, intestine and duodenum were weighed on the 18th day of incubation. Following duodenal grafts the weight of the whole chick decreased while the weight of the spleen, liver and heart significantly increased in weight. The weight of the liver and heart were found to be increased subsequent to liver grafts. When heart was grafted no weight increases were found in the host heart, spleen, liver and intestine. Following grafts of adult spleen the spleen and heart were found to be enlarged. The spleen, liver and heart were enlarged following grafts of brain tissue. When skin was grafted to the CAM the spleen, liver and duodenum were enlarged. Further, when embryonic duodenum was grafted no significant changes were observed. The degree of enlargement of the various host organs was significantly greater following grafts of adult duodenum than all other treatments. The relative weight of the duodenum was found to be increased following duodenal grafts.

Grafting of adult duodenum caused acceleration of tissue differentiation of the host duodenum. Following grafting of duodenum, spleen and skin the host spleen exhibited a marked increase in granuloblasts and granulocytes. The heart and liver following grafts were essentially like those in control embryos. The polysaccharides in the connective tissue and goblet cells of the duodenum following CA grafts of adult duodenum, differentiated at least 24 hours earlier than in control chicks.

An immunological study of the embryonic duodenum from 11 through 21 days of incubation was carried out using the Ouchterlony agar diffusion plate technique. Antiserum against adult chicken duodenum was prepared and saline soluble antigens of the various ages of duodena were tested. Two lines were observed in 11-, 12- and 13-day duodena; a third line in the 14-day duodenum; four lines in 15-, 16- and 17-day duodena; with only three lines in 18-, 19- and 20-day duodena and by the 21st day there were four lines. The Björklund inhibition technique was used to demonstrate that the fourth line in the 15-, 16-, 17-day and in the 21-day duodena was not found in the 18-day duodenum. Further, this method was also used to demonstrate that the three lines in 20-day and three of the four lines in 21-day duodena were not serum antigens.

The agar diffusion procedure was also used to demonstrate changes in the antigenic pattern of the 17-day

embryonic duodenum and spleen following CA grafts of adult duodenum and spleen. Following spleen grafts no qualitative changes were observed in the spleen but an extra line was observed in the duodenum. One extra line was observed in the spleen and three in the duodenum subsequent to grafts of adult duodenum.

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INTRODUCTION

The grafting of tissues to the chorioallantoic membrane (CAM) of the chick embryo has been widely used for study of organ-specific growth stimulation. Murphy (1916) observed that chorioallantoic (CA) grafts of adult chicken spleen, liver, kidney and bone marrow induced enlargement of homologous organs of host embryos, e.g., a striking enlargement of the host spleen following grafts of adult spleen. Spleen hypertrophy was attributed to increased infiltration of small lymphocytes into and confluent with the transplanted tissue. Further, clumps of both granular and nongranular cells were observed adjacent to blood vessels of the liver and kidney. These observations were confirmed by Danchakoff (1916) although she attributed spleen hypertrophy to an intense proliferation of lymphoid hemocytoblasts which ultimately differentiated into granulocytes. In a subsequent study Danchakoff (1918) observed that transformation of mesenchyme into granuloblastic cells was not confined to the spleen but extended throughout the whole mesenchyme of the host. Minoura (1921) transplanted ovarian and testicular tissue to the CAM and observed that development and differentiation of one sex was stimulated by secretions of the gonads of the same sex and inhibited by the gonadal secretions of the opposite sex.

In a study to determine the effects of thyroid tissue on development of chick embryos, Willier (1924) observed that CA thyroid grafts not only induced proliferation of leucocytes but frequently induced conspicuous necrotic nodules in the spleen and to a lesser extent in liver and skin. This hypertrophy of the embryonic chick spleen was also observed following spleen, liver and thymus gland grafts. When using embryonic tissue (organ primordia) he was unable to find any apparent effects on the host embryo. Sandstrom (1932) also observed host spleen hypertrophy following grafting of adult chicken spleen. He attributed hypertrophy to proliferation of granulocytes within the host spleen.

In recent years four views have been advanced to explain growth and differentiation, i.e., the template-antitemplate hypothesis of Weiss (1947), the natural auto-antibody concept of Tyler (1947), the hierarchy of self-limiting reaction hypothesis of Rose (1952), and the building block hypothesis of Ebert (1954).

Weiss (1952, 1953 a, b) and Weiss and Kavanau (1957) maintained that protoplasmic synthesis of a given organ yields: (a) molecular "templates" for further development of specific organ molecules and (b) accessory diffusible compounds ("antitemplates") antagonistic to the former which block and thus inhibit the reproductive activity of the corresponding

"templates". This concept when first proposed, was based on observations which demonstrated that liver and kidney of chick embryos hypertrophied following homoplastic grafts of corresponding organs (Weiss and Wang, 1941). Weiss, (1955) observed that addition of kidney extract to tissue cultures of metanephros fragments reduced the frequency of tubule differentiation. In experiments with heart cultures he observed that heart extract depressed differentiation of cardiac fibrils, as evidenced by reduction in frequency of pulsation. Since differentiation was inversely related to cell proliferation he concluded that substances in cell extracts promoted homologous growth either by being incorporated into the corresponding cells or by neutralizing homologous growth inhibitors of the medium. In a subsequent study Weiss and Andres (1952) showed that presumptive melanoblasts of dissociated embryonic cell suspensions, when injected into the blood stream of early chick embryos, localized in the regions characteristic of the cell type of the donor strain. Andres (1955) who extended the experiments, showed that intravascular administration of mesonephros and liver material had a specific stimulation effect on proliferation in homologous organs. The observation of Pomerat (1949) supported this view since he found an enormous increase in the size of embryonic spleen following CA grafts of adult spleen.

The natural auto-antibody hypothesis proposed by Tyler (1947) was based mainly upon the observation that both fertilizin and antifertilizin can be found in the sea-urchin egg.

The concept of a hierarchy of self-limiting reactions was proposed by Rose (1952) and this generalization was based on regeneration experiments made with Tubularia. Further, in four out of twenty-six experiments in which he cultivated frog eggs in the presence of fragments of adult brain and heart, he observed a specific inhibition of the developing homologous organ (Rose, 1955). The experiments of Shaver (1954), in which he observed that a granular fraction of adult frog brain arrested brain differentiation, also lend support to this view.

An extensive investigation of the problem of the effect of CA grafts of adult chicken tissue on homologous tissues of the host embryo was carried out by Ebert (1955). He observed a very marked enlargement of spleen in host chicks following grafts of adult chicken spleen (Ebert, 1951). In other experiments of this type, Ebert (1954) observed that nitrogen content of enlarged spleens increased and he concluded that spleen enlargement was directly related to increased protein content. By the use of CA grafts labeled with radioactive methionine Ebert (1954, 1955) was able to demonstrate a higher specific activity in the host spleen

following labeled spleen transplants and a higher specific activity in the host kidney following labeled kidney grafts. Grafts of labeled mouse spleen, in contrast to adult chicken spleen, did not produce any selective incorporation and he concluded that this effect was class-specific as well as tissue-specific.

Ebert (1954) observed that the DNA content of enlarged spleens differed significantly from control spleens. He suggested that there was no transfer of either whole cells or "templates" from grafts to hosts but rather a selective incorporation of tissue-specific proteins from grafts to homologous tissues. A transfer of such specific protein moieties was shown by Walter et al. (1956). Following injection of either a clear supernatant of S^{35} labeled homogenized liver or heart into 9-day old embryos, a higher specific activity was observed in the respective host tissues of liver and heart.

Simonsen (1957) questioned the organ-specific growth stimulation hypothesis. He injected chick embryos incubated for 18 days with adult chicken spleen cells and observed that about 3 weeks post-hatching the chicks manifested symptoms of severe haemolytic anemia and that marked hypertrophy occurred in the spleen, liver and thymus. Further, he attributed replacement of erythropoietic and myelopoietic cells in the bone marrow by proliferating reticulo-endothelial cells to an immunological reaction between donor cells and host tissues.

An extensive review and summary of the early literature on the origin of adult antigens in the developing chick embryo has been made by Needham (1931), Cooper (1946) and Schechtman (1947). Consistent results have been obtained only in recent years by the use of more refined techniques.

Burke, et al. (1944) prepared antisera against saline extracts of adult organs (brain, testis, ovary, kidney, liver and lens) of the chicken. They maintained that adult organ-specific antigens in the chick embryo appeared subsequent to differentiation and development of the organ, e.g., eye lens at 146 hours, erythrocytes at 100 hours, kidney at 220 hours, and brain, testis and ovary at 260 hours. On the other hand, Schechtman (1948) and Ebert (1950) maintained that antigens of the brain, heart and spleen were identifiable in the early chick blastoderm. Furthermore, lens antigens were identified in the chick at 60 hours of incubation by Ten Cate and Van Doorenmaalen (1950) and Flickinger et al. (1955).

Serum-like antigens were identified in the egg yolk and in extracts of various ages of chick embryos. Further antigen activity was reduced by absorption with anti-adult serum, anti-albumin and anti-γ-globulin (Schechtman 1947, Nace and Schechtman 1948). Similar results were obtained by Schechtman and Hoffman (1952) using anti-α β- globulin serum. In further work Nace (1953) observed that serum of

chick embryos exhibited a vitelloid albumin present from the third day, a nonvitelloid albumin by the 5th day, and vitelloid γ-globulin was detected by the 9th day and a nonvitelloid γ- globulin was not present until the 12th day and a nonvitelloid $\alpha\beta$-globulin was present from the 6th day. He suggested that the yolk proteins may be a direct transudate from the maternal serum. Tyler (1957), in an alternative explanation for the reduction in reactivity of the antiserum of adult chicken blood following absorption with yolk proteins, attributed this to similar antigenic determinant groups in the yolk but not necessarily to the particular protein they were formed against.

Schechtman (1948) was the first to identify an organ antigen in the early chick embryo, i.e., primitive streak, early neurula and 4- to 5-somite stage. He prepared antisera against a saline extract of perfused brain of chicks of 19 to 20 days of incubation and observed that these antisera cross-reacted with extracts of liver, heart, muscle and blood of chicks the same age. Following absorption with blood the antisera no longer reacted with yolk but did react with the various organs and with early embryo extracts. These results have been extended by Ebert (1950, 1951) in which he used anti-brain, anti-heart and anti-spleen sera. He observed that absorption of any of the three antisera with either extracts of heart, brain or spleen removed the

precipitins for definitive primitive streak and early somite stages, while heterologous antigen did not remove precipitins from homologous antigens. Therefore, it appears that the antigen of the early blastoderm was of a more general type than any of the tissue-specific antigens described.

In another study Ebert (1952) was able to demonstrate the ontogeny of tissue antigens in the developing chicken spleen. Antisera were prepared against saline extracts of 18-day embryonic chick spleen and tested with saline extracts of 12- and 18-day embryonic spleen. It was observed that the number of antigens increased from three to six between the 12th and 18th day of incubation. Absorption of anti-18-day spleen sera with 12-day embryonic spleen reduced antigenic reactions from three to zero with 12-day spleen antigens and decreased antigenic reactions with 18-day preparations from six to three.

Immuno-embryological experiments with highly purified cardiac contractile proteins (actin and myosin) in chick embryos have been reported by Ebert (1953, 1955) and Ebert *et al.* (1955). He was able to show that cardiac myosin was not synthesized prior to the mid-primitive streak stage. At this stage the antigen reactive groups of myosin were not localized in any particular region. However, at the head fold stage it became restricted to the heart-forming area and was absent at the head process.

A somewhat similar study of proteins in the chick was made by Johnson and Leone (1955). They detected myogen in extracts of embryos of 40 hours of incubation while actomyosin could only be detected after 40 hours of incubation. Blood proteins (serum albumins and serum globulins) were detected in extracts of 5- to 10-hour blastoderms and extracts of egg yolk and egg albumin.

The histochemistry and histogenesis of the duodenum of the chick embryo has recently been studied extensively. Moog (1950) found an accumulation of alkaline phosphomonesterase in the striated borders and in the crypts of Lieberkühn of the duodenum of 18-day embryos. Moog and Wenger (1952) identified a Schiff-positive material which was difficult to hydrolyze, was resistent to diastase and hyaluronidase, and was not metachromatic at sites of high alkaline phosphatase activity. Esterase was identified by Richardson et al. (1955) in the connective tissues and epithelium of the duodenum of 10-day embryos, while in later stages it was observed only in the epithelium. Esterase was found to gradually increase between 9 and 17 days and then it rapidly increased and remained at a high level through 3 days post-hatching at which time it began decreasing. Recently, Kato and Moog (1958) have demonstrated an increase in the amount of alkaline phosphatase in the 17-day duodenum following injection of disodium phenylphosphate into chick embryos on the 14th day.

The histogenesis of the duodenum of the chick has been described by Van Alten and Fennell (1957). They also described the histochemistry of the development of mucopolysaccharides in the connective tissues of this organ, and observed a Schiff-positive material on the 18th day of incubation which they attributed to hyaluronic acid.

The above review of literature would indicate that an organ-specific growth stimulating material is produced following CA grafts of adult tissue. It would also seem possible that by means of immunological techniques one would be able to detect adult antigens being transferred from the grafts to the organs.

The objectives of this investigation were to study: (1) the effects of chorioallantoic grafts of embryonic duodenum and various adult organs (spleen, liver, heart, skin, brain and duodenum) upon homologous and heterologous organs in the developing chick embryo; (2) by means of immunological technique the difference in antigens of adult, embryo and embryos treated with adult spleen and duodenum grafts; and (3) the histology of the various embryonic organs following CA transplants.

MATERIALS AND METHODS

Hatching eggs of single-comb white leghorn chickens were used exclusively in these studies. The tissues used for grafting experiments were obtained from various breeds and ages of adult chickens.

The eggs were incubated at 37.5° C in a moistened air, constant rotating, forced circulation incubator for 9 days prior to chorioallantoic grafting. Subsequent to grafting the eggs were incubated at 37.5° C in a nonrotating, moistened air incubator. All weight studies were made on chicks which were incubated 18 days, just prior to the absorption of the yolk sac.

On the 9th day of incubation the eggs were candled over a 75-Watt electric bulb. An area near the bifurcation of two large blood vessels was marked by swabbing with tincture of merthiolate 1:1000 which was also used for sterilization of the shell. Prior to candling, the air sac end of the egg was also swabbed with tincture of merthiolate. Subsequently, a small hole was drilled by means of a motor-driven burr into the air sac and a second was carefully drilled through the shell to expose the shell membrane above the bifurcated vessels. A small sterile needle was used to puncture the shell membrane. Care was taken not to puncture or tear the underlying vascular

chorioallantoic membrane. Simultaneously decreasing the pressure at the air sac caused the CAM to drop away from the shell membrane thus displacing the air sac. The hole over the CAM was enlarged by very carefully removing the shell with a pair of sharply pointed forceps.

Chickens of both sexes, of different breeds and of ages ranging from 3 months to about one year post-hatching were used as donors of tissues for grafting. The chicken was killed by removing the head and various tissues (duodenum, spleen, liver, heart, skin and brain) were quickly removed and cut into very small pieces in chick Ringer's solution. In order to sterilize the duodenum it was placed in 200 ml of chick Ringer's solution containing 1000 mg of chloromycetin for 10 minutes. One of the prepared pieces of tissue, 2-3 mm, was then placed at the bifurcation point of the blood vessels on the CAM. The hole through which tissues were inserted were sealed with Scotch tape and melted paraffin and the hole leading into the former air sac was sealed with just the melted paraffin. The egg was then placed in the incubator with the window faced upward. The eggs continued to incubate until the 18th day when embryos and various vital organs were weighed and some were preserved for further study. The above procedure was also used for duodenal grafts of 15-, 16-, 17-, 18- and 20-day embryos and for adult rat duodenum. Controls

of two types were used, either a drop of Ringer's solution or a piece of 2% agar were placed on the CAM.

In order to ascertain the effect of soluble cell free material of the duodenum, liver and heart of the adult chicken on the chick embryo the following procedure was carried out. The adult chicken was killed by decapitation and the above tissues were removed under sterile conditions and put into cold sterile saline. The duodenum was opened, thoroughly washed and then treated in a solution of 1000 mg of chloromycetin in 200 ml of 0.15 M saline. The adult tissue was homogenized in a Waring blender with a 1 to 5 ratio of 0.15 M saline (buffered to pH 7.4 with 0.005 M phosphate buffer). It was homogenized for 20 minutes at 4° C and then centrifuged at 2000 RPM for 30 minutes at 4° C. The supernatant was used for injection into the yolk sac. Eggs which had been incubated for 96 hours were candled and the shell was swabbed with tincture of merthiolate just below the equator. At this site a small hole was drilled through the shell but not through the shell membrane by means of a motor-driven burr, so that a 23-gauge needle could be thrust into the yolk sac. Eggs were injected by means of a 1cc tuberculin syringe and 0.1cc of adult tissue homogenate or 0.15 M saline was used. The hole was closed with melted paraffin and the eggs were returned to the incubator but were candled every day and dead

ones were removed. Various dilutions of the stock antigens were prepared and injected by the same method.

The agar diffusion technique of Ouchterlony (1949) (which has recently been reviewed by Oudin, 1952) was used in the study of adult antigen formation in the duodenum (11 through 21 days of incubation) and for comparison of 17-day spleens and duodena following CA grafts of adult duodenum and adult spleen. All tests were made in 90 mm Petri dishes which were prepared in the following manner. A 2% agar solution in 0.85% saline buffered with 0.005 M phosphate buffer at pH 7.4 was prepared, filtered and measured out into 30 ml quantities. Eight 1 5/8 inches x 1/2 inch strips of filter paper (Whatman #1) were arranged equidistant around the lip of the male half Petri dish with a small portion lying on the bottom of the dish. A stainless steel wire was placed inside the male half Petri dish securing the paper strips against the wall and bottom of the dish. The dish was then sterilized by dry heat at 120° C for 12 hours. Following sterilization 30 ml of sterile agar werepoured into the male half of the dish and the agar was allowed to harden. After hardening, a template was placed over the plate, the wells were cut and the pieces of agar left in the wells were removed (fig. 1). A drop of agar was then placed in each well to seal the bottom. Each of the above wells held 0.15 ml of test solution. Due to the

difficulty in analyzing complex systems containing numerous antigenic components the Björklund (1952) specific inhibition technique was employed. In this case the antigen which was used for inhibition was placed in the antiserum well (fig. 1) in doses of 0.15 ml until the desired number of inhibiting doses were absorbed. Subsequent to the last dose the plates were set aside for 48 hours before addition of the test antigen and antiserum.

Adult chicken duodenum (from which the pancreas had been removed), liver, heart, spleen and brain were used for preparing antigens. These organs were removed from several adult chickens ranging in age from 3 months to 1 year. The organs were drained on several thicknesses of filter paper, weighed, mixed with 5 ml of cold 0.15 M saline (buffered to pH 7.4 with 0.005 M phosphate buffer) per gram of tissue, homogenized in a Waring blender for 10 minutes and further homogenized with a glass homogenizer for 30 minutes at 4° C. The homogenate was centrifuged at 4° C for 30 minutes at 2000 RPM and frozen until ready to use. Antigens of embryonic chick duodena of 11 to 21 days inclusive (from which the pancreas had been removed) and spleens of 17-day embryos were prepared in a similar manner except that all homogenizing was done with only a glass homogenizer.

Antisera were prepared against adult chicken duodenum and spleen by injecting the pooled antigen into three male rabbits. Five intravenous injections were given during a 10-day period, one every other day. The first two injections were 0.5 ml of antigen each; the third was 1 ml, the fourth 1.5 ml and the fifth was 2 ml. The rabbits were rested for two weeks following the injections and then given an anamnestic injection of 2 ml of antigen intravenously. Seven days later the rabbits were bled and a precipitin titer was run. If the titer was at least 1:64 the rabbit was bled on the following day, sera collected, 1:5000 merthiolate added, complement inactivated by heating at 56° C for 30 minutes and the serum was frozen until used. If the titer was not 1:64, the rabbit was given another 2cc injection of antigen on the following day and a titer was run again in another 8 days. This procedure was carried out until a sufficient titer was obtained.

The statistics used in comparing the weight of the chick, spleen, liver, heart, intestine and duodenum following various treatments to the CAM, were an analysis of variance (Dixon and Massey, 1951) and the multiple range test for heteroscedastic means (Duncan, 1957). The former test, analysis of variance, is based upon the fact that if means of groups are greatly different the variance of the combined groups is much larger than the variance of the

separate groups. However, an F test alone, when it rejects the homogeneity hypothesis, gives no decisions as to which differences among the treatments may be considered significant and which are not. The purpose of using the multiple range test was to designate which differences among the treatment means might be considered significant and which were not.

Duncan (1957) proposed the following basic rule for an α-level multiple range test. "Any subset of P means is homogeneous if the largest adjusted difference in the subset fails to exceed the critical value R'P. Any two means not both contained in the same homogeneous subset are significantly different. Any two means both contained in the same homogeneous subset are not significantly different." To illustrate the procedure used, Table I shows the processing of some data obtained from one of the experiments.

Following various CA grafts, tissues of spleen, liver, heart, duodenum and CAM were fixed in Bouin's fixative for 24 hours. Tissues were then transferred to 70% alcohol (5 changes of 24 hours each), dehydrated and embedded in paraffin by routine methods of tissue preparation. Tissue sections were cut at 5 microns and affixed to clean glass slides. All tissues were routinely stained with hematoxylin and eosin, Gomori's trichrome stain (Gomori, 1950), the triple stain for DNA polysaccharides and proteins of

TABLE 1

MULTIPLE RANGE TEST

a) Analysis of Varience

Source	d.f.	Sum Squares	Mean Squares
Total	107	501.04	
Between	3	107.20	35.73 F = 9.41
Within	104	394.84	3.797 S = $\sqrt{M.S.}$ = 1.9486

b) Ranked Treatment Means and Replication Numbers

	A	D	B	C
Mean	16.25	17.60	18.13	18.86
n	(33)	(21)	(29)	(25)

c) Critical Values: R'p = S*Zp

P:	(2)	(3)	(4)
Zp[1]	2.80	2.95	3.05
R'p	5.4561	5.7484	5.9432

d) Arithmetical Details for Calculating Adjusted Differences

$(C-A)' = (C-A)^{A}AC = 2.61\sqrt{2(33)(25)/58} = 13.92096$

Similarly:

$(C-D)' = 1.26(4.7777) = 6.01990$ $(B-A)' = 1.88(5.5562) = 10.4457$

$(C-B)' = 0.73(5.1819) = 3.78279$ $(D-A)' = 1.40(5.0662) = 7.0927$

$(B-D)' = 0.53(4.9356) = 2.61587$

e) Test Sequence

$(C-A)' > R'4$, $(C-D)' > R'3$, $(C-B)' \not> R'2$

$(B-A)' > R'3$, $(B-D)' \not> R'2$

$(D-A)' > R'2$

Results: (BC) (BD)

f) Result

(BC) (BD)

Any two means appearing together within the same parenthesis are not significantly different at the 5% level. Any two means not appearing together within the same parenthesis are significantly different.

[1]Values for Zp are obtained from tables in Duncan (1955).

Himes and Moriber (1955) and the Azure II eosin procedure (Lillie, 1954). For elastic tissue Weigert's resorcin fuchsin was used and Van Giesen's picro-acid fuchsin was used for collagenous tissue.

RESULTS

1. THE EFFECT OF CHORIOALLANTOIC TRANSPLANTS OF ADULT AND EMBRYONIC CHICKEN TISSUES ON THE WEIGHT OF HOMOLOGOUS AND HETEROLOGOUS TISSUES OF THE HOST EMBRYO.

It is evident from Table 2 that following adult duodenal grafts there was a significant reduction in weight of the whole embryo, a marked enlargement of the spleen, liver and heart and that there was no significant weight difference in the intestine or duodenum. It can also be seen that subsequent to liver grafts the liver and heart showed a significant enlargement over those of controls (sham operation). However, the liver and heart were significantly larger subsequent to grafts of duodenum than those following liver grafts. When alcohol inactivated adult chicken duodenum was transplanted to the CAM it was observed that there was a decrease in weight of both the whole embryo and the duodenum.

It is evident from the multiple range test in Table 3 that the weight of the whole chick significantly decreased while the spleen, liver, heart and duodenum all exhibited a significant increase in weight following grafts of adult duodenum. It was further observed that following heart grafts there was a significant increase in weight of both

TABLE 2

FRESH WEIGHTS OF WHOLE EMBRYOS AND OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host and Host Organs: Whole Chick Grams	Spleen mg	Liver Grams	Heart Grams	Intestine Grams	Duodenum mg
A. Grafts of adult chicken duodenum	33	16.25	36.41	0.6609	0.2115	0.3963	67.08
B. Grafts of adult chicken liver	29	18.13	16.97	0.5421	0.1786	0.4148	67.08
C. Sham operation	25	18.86	12.00	0.4716	0.1592	0.3924	61.96
D. Grafts of inactivated adult chicken duodenum	21	17.60	13.195	0.4514	0.1448	0.3757	55.37
Statistical F Test		9.41*	22.19*	30.77*	28.75*	0.75	5.72*
Statistical multiple range test ‡		(BC)(BD)	(BCD)	(CD)	(CD)	(ABCD)	(ABC)(CD)

‡ Any two means appearing together within the same parenthesis are <u>not</u> significantly different at 5% level.

TABLE 3

FRESH WEIGHTS OF WHOLE EMBRYOS AND OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host and Host Organs: Whole Chick Grams	Spleen mg	Liver Grams	Heart Grams	Intestine Grams	Duodenum mg
A. Grafts of adult chicken duodenum	25	20.91	32.08	0.7060	0.2100	0.5588	76.08
B. Grafts of adult chicken heart	21	24.26	13.11	0.5657	0.1852	0.6781	81.54
C. Grafts of inactivated adult chicken duodenum	20	22.11	12.03	0.5060	0.1580	0.5410	56.34
D. Sham operation	23	23.47	11.77	0.5478	0.1726	0.5839	65.33
Statistical F test		13.09*	28.37*	16.36*	16.67*	11.43*	21.46*
Statistical multiple range test ‡		(BD)	(BCD)	(BCD)	(BD)(CD)	(ACD)	(AB)

‡ Any two means appearing together within the same parenthesis are <u>not</u> significantly different at 5% level.

the intestine and duodenum. A comparison of the observations in Table 8 with those in Table 3 shows that in the former the weights of the intestine and duodenum differ from those of the latter and are not always significantly larger. Transplantation of alcohol inactivated chicken duodenum induced a significant decrease in the weight of the whole embryo and the duodenum (Table 3).

From the multiple range test as given in Table 4 it is seen that subsequent to duodenal transplants the spleen, liver, heart, and duodenum showed a significant enlargement. When adult spleen was grafted the host spleens were significantly larger than control spleens but were still significantly smaller than the spleens subsequent to duodenal grafts.

In Table 5 it is evident that the spleen and heart were significantly larger when brain was grafted to the CAM than when control procedures were used. The duodenum was larger but other significant growth changes were not observed following grafts of rat duodenum.

It is evident on the basis of the multiple range test (Table 6)that following duodenal grafts the weight of the whole embryo was decreased significantly and the weights of the spleen, liver and heart were significantly increased in contrast to decreased weights of these organs in controls and following lyophilized duodenal grafts. On the other

TABLE 4

FRESH WEIGHTS OF WHOLE EMBRYOS AND OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host and Host Organs: Whole Chick Grams	Spleen mg	Liver Grams	Heart Grams	Intestine Grams	Duodenum mg
A. Grafts of adult chicken duodenum	22	18.93	45.61	0.7068	0.2314	0.5105	71.93
B. Grafts of adult chicken spleen	35	17.71	24.13	0.5440	0.1631	0.4772	54.27
C. Grafts of inactivated chicken duodenum	24	19.60	11.52	0.4850	0.1479	0.4675	59.87
D. Sham operation	26	21.23	12.35	0.5035	0.1546	0.5619	58.70
Statistical F test		2.25	25.35*	28.75*	37*	9.4*	13.88*
Statistical multiple range test ±		(ABCD)	(CD)	(BD)(CD)	(BCD)	(ABC)	(BCD)

± Any two means appearing together within the same parenthesis are <u>not</u> significantly different at 5% level.

TABLE 5

FRESH WEIGHTS OF WHOLE EMBRYOS AND OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host and Host Organs					
		Whole Chick Grams	Spleen mg	Liver Grams	Heart Grams	Intestine Grams	Duodenum mg
A. Grafts of adult chicken duodenum	19	20.63	54.66	0.7158	0.2121	0.5778	58.73
B. Grafts of adult chicken brain	28	23.26	17.49	0.5681	0.2026	0.6143	58.60
C. Grafts of adult rat duodenum	21	22.58	9.89	0.5267	0.1590	0.5700	62.93
D. Sham operation	26	22.53	9.83	0.5142	0.1546	0.5692	55.77
Statistical F test		7.05*	75.91*	24.29*	5.67*	1.43	3.12*
Statistical multiple range test ‡		(BCD)	(CD)	(BC)(CD)	(AB)(CD)	(ABCD)	(ABC)(ABD)

‡ Any two means appearing together within the same parenthesis are <u>not</u> significantly different at the 5% level.

TABLE 6

FRESH WEIGHTS OF WHOLE EMBRYOS AND OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host and Host Organs: Whole Chick Grams	Spleen mg	Liver Grams	Heart Grams	Intestine Grams	Duodenum mg
A. Grafts of adult chicken duodenum	20	21.36	43.31	0.7280	0.2405	0.6285	64.72
B. Grafts of adult chicken lyophilized duodenum	38	23.16	13.89	0.5253	0.1861	0.5737	54.19
C. Sham operation	28	23.35	13.10	0.5393	0.1636	0.5925	64.06
Statistical F test		4.86*	103.21*	36.88*	14.29*	3.33	10.52*
Statistical multiple range test ‡		(BC)	(BC)	(BC)		(ABC)	(AC)

‡ Any two means appearing together within the same parenthesis are not significantly different at 5% level.

hand, when lyophilized duodenum was placed on the CAM the weight of the host heart was significantly higher while the duodenum was significantly lower.

On the basis of evidence presented in Table 7 it can be seen that when adult chicken duodenum was placed on the CAM there was a significant decrease in the weight of the whole chick and a significant increase in the weight of individual organs, i.e., spleen, liver and heart. Following grafts of either heated or alcohol extracted chicken duodenum there were no significant weight changes observed in either the weight of the whole embryo or the individual organs.

Table 8 shows that on the basis of the multiple range test there was no significant differences observed in the weights of whole chicks or of individual organs following heart grafts. Further, following the grafting of skin the multiple range test shows that spleen, liver and duodenum had significantly increased in weight.

The question naturally arises as to whether enlargement of host embryonic organs following grafts of adult tissue was dependent on an increase in protoplasm or was merely a manifestation of edema. The following analysis indicates that the enlargement was due to an increase in protoplasm. It is evident in Table 9 that the fresh and dry weights of the spleen and heart were significantly

TABLE 7

FRESH WEIGHTS OF WHOLE EMBRYOS AND OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host and Host Organs: Whole Chick Grams	Spleen mg	Liver Grams	Heart Grams	Intestine Grams	Duodenum mg
A. Grafts of adult chicken duodenum	34	23.45	46.63	0.7229	0.2185	0.6412	66.51
B. Grafts of heated adult chicken duodenum (80° C for 20 minutes)	30	24.85	13.22	0.5713	0.1917	0.6607	73.43
C. Grafts of alcohol extract of adult chicken duodenum	36	24.41	12.38	0.5494	0.1853	0.6142	66.64
D. Sham operation	27	24.81	12.05	0.5378	0.1800	0.6622	71.77
Statistical F test		4.09*	36.46*	25.77*	8.75*	2.13	3.95*
Statistical multiple range test ‡		(BCD)	(BCD)	(BCD)	(BCD)	(ABCD)	(AC)(BD)

‡ Any two means appearing together within the same parenthesis are not significantly different at 5% level.

TABLE 8

FRESH WEIGHTS OF WHOLE EMBRYOS AND OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host and Host Organs: Whole Chick Grams	Spleen mg	Liver Grams	Heart Grams	Intestine Grams	Duodenum mg
A. Grafts of adult chicken heart	19	24.89	16.06	0.5684	0.1763	0.6221	71.11
B. Grafts of adult chicken skin	25	24.97	28.86	0.6540	0.1868	0.6688	82.32
C. Sham operation	35	25.32	14.07	0.5640	0.1871	0.6737	72.11
Statistical F test		0.37	7.54*	6.25*	1.85	2.14	3.38*
Statistical multiple range test ‡		(ABC)	(AC)	(AC)	(ABC)	(ABC)	(AC)

‡ Any two means appearing together within the same parenthesis are not significantly different at 5% level.

TABLE 9

FRESH AND DRY WEIGHTS OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host Organs							
		Spleen Fresh mg	Spleen Dry mg	Liver Fresh Grams	Liver Dry Grams	Heart Fresh Grams	Heart Dry Grams	Duodenum Fresh mg	Duodenum Dry mg
A. Grafts of adult chick-duodenum	25	32.65	9.66	.7014	.1598	.1733	24.84	91.83	12.58
B. Grafts of adult chick-spleen	25	24.04	5.06	.5930	.1383	.1515	21.75	90.62	11.56
C. Sham operation	28	13.37	2.33	.5909	.1424	.1250	17.97	103.66	12.49
Statistical F test		21.69*	4.44*	139.57*	5.29*	45.79*	25.64*	3.71*	2.77
Statistical multiple range test ‡				(BC)	(BC)			(AB)	(ABC)

‡ Any two means appearing together within the same parenthesis are <u>not</u> significantly different at 5% level.

larger following spleen and duodenal grafts and it also shows that following duodenal grafts the fresh and dry weights of the liver were also significantly increased. The dry weight of the duodenum was not significantly different from the controls following either duodenal or spleen grafts. Of the weight increases induced by the grafting of duodenum or spleen, the former produced the greatest weight increase of host organs.

It is evident in Table 2 that the weight of the whole chick following duodenal grafts was lower than that of control embryos. It is also evident that on the basis of the multiple range test the weight of the duodenum in both the control and experimental (duodenal) grafts was not significantly different. The question arises as to whether the relative weights of duodena following duodenal grafts are significantly higher than those of the controls.

The covariance test was used to ascertain whether the weight of the whole chick had any effect on the weight of the duodenum. The F_1 value given in Table 10 measures the difference between the two sample regression coefficients. This value is 6.6 with a df. = 1, 51 and at the 95% level this is significant. This clearly indicates that the weight of the duodenum following duodenal grafts is statistically larger than following control procedures.

TABLE 10

COVARIANCE TEST ON THE WEIGHT OF THE DUODENUM TO THE WEIGHT OF THE WHOLE EMBRYO

Line	Treatment	df.	ξx^2	ξxy	ξy^2	Deviations from Regressions df.	$\xi dy \cdot x^2$	Mean Square	Regression Coeff.
1	Control	24	49.98	0.1235	0.60249	23	0.00218	0.00009	0.00247
2	Duodenum graft	29	97.28	0.3809	0.00449	28	0.00300	0.000107	0.00392
3	Within					51	0.00518	0.000101	
4	Regression coefficient					1	0.00067	0.00067	
5	Common	53	147.26	0.5044	0.00758	52	0.00585	0.00011	0.00343
6	Adjusted Mean					1	0.00182	0.00182	
7	Total	54	147.11	0.23257	0.00804	53	0.00767		

$$F_1 = \frac{0.00067}{0.000101} = 6.6^*, \text{ df.} = 1, 51$$

Since transplantation of adult tissue to the CAM induces profound changes in various organs of the embryo, the question arises as to whether or not grafts of embryonic tissue would have the same effect as adult tissues on host organs. Since the adult duodenum was the most effective stimulant for host tissues, duodena from various ages of embryos were transplanted to the CAM.

The multiple range test in Table 11 shows that the weight of the whole chick following grafts of 16-day embryonic duodenum is significantly higher than the weight of embryos following grafts of 18- and 20-day duodena but is not significantly different from control experiments. However, the weight of the whole chick following 18-day embryonic duodenal grafts is significantly less than the controls. Further, the weight of the spleen, liver, heart and intestine are not significantly higher following grafts of either 16-, 18- or 20-day embryonic duodenum from that of the control. However, it is evident that following grafting of 20-day chick embryonic duodenum the weight of the duodenum is significantly decreased while weights of duodena following grafting of 18- and 16-day duodenum were not significantly different from controls.

It is evident in Table 12 that the multiple range test shows that the weight of the whole chick is not significantly different from that of the control following grafts of 15-, 16- and 17-day chick embryonic duodena. Further,

TABLE 11

FRESH WEIGHTS OF WHOLE EMBRYOS AND OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host and Host Organs: Whole Chick Grams	Spleen mg	Liver Grams	Heart Grams	Intestine Grams	Duodenum mg
A. Grafts of 16-day chick embryo duodenum	24	26.13	13.47	0.5908	0.1817	0.6613	74.65
B. Grafts of 18-day chick embryo duodenum	24	24.66	13.05	0.5717	0.1796	0.6658	67.70
C. Grafts of 20-day chick embryo duodenum	37	24.86	12.72	0.5630	0.1768	0.6286	60.88
D. Sham operation	22	25.56	11.62	0.5577	0.1709	0.6541	71.39
Statistical F test		5.07*	1.62	2.33	0	0.875	8.75*
Statistical multiple range test ‡		(AD)(CD)(BC)	(ABCD)	(ABCD)	(ABCD)	(ABCD)	(AD)(BD)

‡ Any two means appearing together within the same parenthesis are <u>not</u> significantly different at 5% level.

TABLE 12

FRESH WEIGHTS OF WHOLE EMBRYOS AND OF HOMOLOGOUS AND HETEROLOGOUS ORGANS FOLLOWING CHORIOALLANTOIC TRANSPLANTS

Treatment	No. of Cases	Mean Weight of Host and Host Organs: Whole Chick Grams	Spleen mg	Liver Grams	Heart Grams	Intestine Grams	Duodenum mg
A. Grafts of 15-day chick embryo duodenum	33	25.25	14.25	0.5752	0.1733	0.6621	71.86
B. Grafts of 16-day chick embryo duodenum	25	25.14	17.86	0.5840	0.1820	0.6496	74.84
C. Grafts of 17-day chick embryo duodenum	34	24.75	16.36	0.5644	0.1724	0.6526	65.85
D. Sham operation	26	24.71	18.34	0.5585	0.1742	0.6788	68.13
Statistical F Test		0.32	5.1*	2.33	1.67	1.11	2.81*
Statistical multiple range test ‡		(ABCD)	(AC)(BCD)	(ABCD)	(ABCD)	(ABCD)	(ACD)(ABD)

‡ Any two means appearing together within the same parenthesis are <u>not</u> significantly different at 5% level.

the weights of liver, heart and intestine in each experimental procedure were not significantly different from controls. On the other hand, the weight of the spleen of embryos receiving 15-day chick embryonic duodenum was significantly lower than the controls. The duodenum following 16-day embryonic duodenal grafts was significantly greater than the weight of duodena obtained with 17-day embryonic duodenal grafts.

During the course of this investigation it was observed that subjection of adult chicken duodenum prior to transplantation to either 95% alcohol at -20° C for 24 hours, lyophilization, a temperature of 80° C for 20 minutes or immersion into 1:10,000 merthiolate in 0.15 M sodium chloride for 30 minutes, host tissues did not respond to the stimulus.

2. THE EFFECT OF SOLUBLE ANTIGENS OF ADULT AND EMBRYONIC CHICKEN ORGANS ON THE HOST EMBRYO WHEN INJECTED INTO THE YOLK SAC

Soluble antigens of 0.1 ml of adult chicken duodenum, liver and heart were injected into the yolk sac of 96-hour embryos while controls were injected with 0.1 ml of 0.15 M saline. A mortality rate of 100% was observed following the injection of supernatant of homogenized duodenum. The time of death varied with the strength of the injection. The results of this experiment have been summarized in Table 13.

TABLE 13

THE EFFECT OF SOLUBLE ANTIGENS ON THE HOST EMBRYO WHEN INJECTED INTO THE YOLK SAC

Amount mg wet wt.	Time death occurred
20 mg	24 hours
10 mg	36-48 hours
7.7 mg	48-60 hours
6.7 mg	56-72 hours

Similar results, i.e., all embryos died, were observed following the injection of adult chicken liver and heart and 20-day embryonic duodenal soluble proteins. Death of the embryo subsequent to injection with these soluble materials could not be attributed to a pressure effect because the control embryos survived until the 20 th day of incubation at which time they were harvested.

3. MORPHOLOGY OF CHICK EMBRYOS AND VARIOUS HOMOLOGOUS AND HETEROLOGOUS ORGANS SUBSEQUENT TO CHORIOALLANTOIC TRANSPLANTS OF ADULT AND EMBRYONIC CHICKEN TISSUES

Whole embryo. It was observed that when embryos were harvested on the 18th day of incubation subsequent to grafting of adult chicken duodenum there was a marked decrease in the size of the chick and it also appeared pale, ashen and anemic. When adult chicken spleen was transplanted to the CAM there were a few chicks which appeared pale but none as marked as in the case of the duodenal transplants.

All embryos appeared normal following CA grafts of adult chicken liver, heart, brain, skin, the various treated duodena, embryonic duodenum and controls.

Duodenum. The gross morphology of the duodenum appeared essentially the same following CA transplants of adult and embryonic chick tissue and control procedures (fig. 2).

On the other hand the differentiation of the microscopic structure of the duodenum was somewhat altered following grafting of adult tissues to the CAM. Following grafting of adult duodenum the connective tissue within the villi consisted of a compact mesenchymal layer until the 15th day and this was succeeded by the lamina propria mucosae on the 16th day. Van Alten and Fennell (1957) under normal conditions observed the lamina propria mucosae on the 17th day. Further, Van Alten (1955) identified goblet cells on the 17th day of incubation in untreated embryos, however, following adult duodenal grafts goblet cells were identified in great abundance on the 16th day of incubation. These observations indicate that duodenal grafts accelerated differentiation of the duodenal tissues of the host embryo.

It appeared that following grafting of adult duodenum there were more goblet cells in the host duodenum than in the control duodenum. Table 14 shows the number of goblet cells found in host duodena following CA grafts of various tissues and control procedures (all counts were made by

TABLE 14

THE EFFECT OF CHORIOALLANTOIC GRAFTS ON THE DEVELOPMENT OF GOBLET CELLS IN THE CHICK DUODENUM

Treatment to CAM	Mean no. Goblet Cells/6 Fields
A. Adult chicken duodenum	167.00
B. Adult chicken liver	113.83
C. Control	95.50
D. Adult chicken heart	108.50
E. Adult chicken duodenum	120.67
F. Adult chicken spleen	145.00
G. Control	104.33
H. 16-day embryonic duodenum	160.00
I. 20-day embryonic duodenum	83.50
J. 17-day embryonic duodenum	111.83

Statistical F Test = 10.36*

Multiple Range Test:
(ABDEFGHJ), (BCDEFGHJ), (BCDEFGIJ)

averaging three counts per field and counting six different fields of six different sections from the same duodenum). It is evident in Table 14 that the mean number of goblet cells following adult duodenal grafts is 167 and 120.67. On the basis of the multiple range test the higher number of cells, i.e., 167, in the host duodenum is significantly higher than control C but not higher than control G. On the other hand, the number of cells following grafts of adult chicken duodenum E was not significantly higher than controls C and G. These observations indicate that the number of goblet cells following CA transplants of adult chicken duodenum was not consistently greater than those following grafts of other adult or embryonic tissues and control procedures.

During the course of this study other observations show that following grafting of either adult chicken duodenum or spleen there was an infiltration of lymphocytes into the duodenal tissue but this was not observed following liver, heart, brain, skin or embryonic duodenal grafts (fig. 3).

It is evident from the results summarized in Table 15 that transplantation of adult duodenum to the CAM accelerates the histochemical differentiation of both connective tissues and goblet cells in the embryonic duodenum. The muscle connective tissues differentiated on the 13th

TABLE 15

THE DIFFERENTIATION OF POLYSACCHARIDES IN DUODENAL TISSUES FOLLOWING CAM ADULT DUODENAL GRAFTS

Treatment	Muscle Connective Tissue	Lamina Propria Mucosae	Submucosa	Glands and Goblet Cells
	Age of embryo in days			
Control	15	18	18	17
Adult chicken duodenum	13	17	17	16

[1] All tissues were stained by the Hotchkiss-McManus PAS reaction.

day following duodenal grafts while in controls differentiation was not observed until the 15th day. Further, the development of the lamina propria mucosae, submucosa and goblet cells developed 24 hours earlier than in controls.

It is evident from the observations presented in the preceding paragraphs that both morphological and histochemical differentiation were accelerated following grafting of adult chicken duodenum.

Spleen. Spleens of host organs following duodenal grafts were markedly enlarged with a diameter of about 10 mm while the maximum in control embryos was a diameter of 2 mm. The surface of enlarged spleens exhibited numerous white tumor-like protuberances. Essentially the same type of reaction occurred following spleen and skin grafts. On the other hand, control spleens and host spleens following grafts of heart, liver, inactivated duodena and embryonic duodena (15-, 16-, 17-, 18- and 20-day embryos) were spherical and dark reddish-brown in color.

In prepared sections of enlarged spleens of 18-day embryos, which had received CA grafts of either adult chicken duodenum, spleen or skin, marked changes were observed (fig. 5). There were fewer venous sinuses than in the control spleens (fig. 4). Within the pulpa there were few reticular cells, i.e., mesenchymal cells, and many hemocytoblasts were observed. Large nodular areas (as many as

six in a single section) filled with granulocytes were walled off by reticulo-endothelial cells and multinucleated giant cells were found to be quite numerous and uniformly distributed throughout these enlarged spleens. In contrast to control spleens, which contained many erythrocytes within the venous sinuses, very few erythrocytes were observed in enlarged spleens.

It is evident from the above observations that following grafts of adult chicken duodenum, spleen or skin the host embryonic spleen is transformed into a granuloblastic center.

Liver. On the 18th day of incubation the liver consisted of right and left lobes and exhibited what Kingsbury et al. (1956) described as a deep sulfur-yellow color. This was essentially the way the liver also appeared following CA transplants of adult chicken spleen, liver, heart, brain, inactivated duodenum (by heat, lyophilization or alcohol) and embryonic duodenum. Following grafts of adult skin the liver of host embryos (18th day of incubation) was a buff color with small red spots of about 2 mm in diameter uniformly distributed over the entire liver surface. The most marked color change was observed following CA grafts of adult chicken duodenum. The host livers were bile green in color, friable and usually exhibited several relatively large gray areas.

Kingsbury et al. (1956) have described the microscopic structure of the liver parenchyma of the chick embryo as a continuous network of anastomosing tubules delimited by slit-like sinusoids. By the 18th day of incubation in both control and treated embryos lymphocytes existed as small nodules in the connective tissues. Microscopic examination of the enlarged green livers revealed that from a histological point of view they were essentially similar to those of control embryos.

Heart. The heart of 18-day embryos following CA grafts of adult duodenum was pale pinkish-gray in color and appeared to have a length in excess of 10 mm. Hearts of control embryos of the same age had a maximum length of approximately 10 mm and had a bright pink color.

The wall of the heart of the 18-day embryo consisted of three poorly differentiated layers, i.e., the endocardium, myocardium and epicardium. The myocardium, which is the thickest, consisted of a spongy network of muscular bundles. The spaces separating individual bundles of muscle fibers exhibited sparse amounts of tissue fibers. Elastic tissue in the myocardium was confined to the internal surface of the arterioles. Following duodenal grafts the various layers of tissue in the heart of host embryos appeared to be essentially similar to those found in control embryos. Further, other tissues (adult spleen, liver, heart,

skin, brain and embryonic duodenum) when grafted to the CAM did not induce gross or microscopic differences in heart tissues.

Reaction of the Chorioallantoic Membrane. The grafting of small pieces of either adult duodenum or spleen to the CAM induced the development of numerous small tumor-like masses which were sufficiently extensive to cover the major portion of the CAM. Following the grafting of heart tissues to the CAM hemorrhagic regions were frequently observed adjacent to or in the vicinity of the grafted tissue of host embryos on the 18th day of incubation.

A microscopic examination of the CAM adjacent to the graft site, i.e., grafts of adult duodenum, spleen, liver, heart and skin, exhibited an abundance of granuloblasts and granulocytes. The cytoplasm of these cells exhibited an abundance of eosinophilic granules. Many basophilic cells were also observed in the graft region and these cells were identified as hemocytoblasts. In almost every instance there were foci of grafted tissue which had not survived and these appeared as an acidophilic mass with numerous basophilic granules (fig. 6).

When embryonic duodenum, i.e., duodenum from 15-, 16-, 17-, 18- and 20-day chicks was transplanted to the CAM the integrity of the original tissue was not destroyed. The

CAM around the graft contained numerous vessels. Within the grafted tissue the mucosa, lamina propria, submucosa and tunica muscularis were identifiable. The epithelium of the mucosa overlying the villi consisted of simple columnar epithelium with numerous functional goblet cells. The lamina propria, which forms the core of the villi, was structurally well differentiated. The muscularis mucosae was in most instances confluent with the circular layer of the tunica muscularis except in areas where the submucosa was definite. The tunica muscularis was seen to consist of two layers, i.e., the inner circular layer and the outer longitudinal layer (fig. 7).

The preceding observations demonstrated that following grafts of adult chicken tissue to the CAM there was a loss of morphological integrity of the original tissue transplanted and in the graft region a myeloid metaplasia occurred. On the other hand, when embryonic duodenum was placed on the CAM the tissue retained its morphological integrity and continued to differentiate.

4. THE APPEARANCE OF ADULT DUODENAL ANTIGENS IN THE DEVELOPING EMBRYONIC DUODENUM OF THE CHICK

Antisera against saline extracts of adult chicken duodenum were tested with saline extracts of adult and 11-through 21-day embryonic chick duodena by a modification of the methods of Ouchterlony (1949) and Björklung (1952).

Figure 8 shows a photograph and diagram of an Ouchterlony plate obtained with anti-adult duodenum serum. This plate consisted of agar with four wells; the lower well contained the antiserum while the other three wells contained saline extracts of 12-, 13- and 14-day embryonic duodenum. In the plate a series of lines of precipitate have developed where there was optimum concentration of antigens and antiserum and these have been numbered 1, 2, and 3 in the diagram.

The results of these experiments show that there were changes in the number of antigens present in the duodenum of the chick embryo between 11 and 21 days of incubation. From the 11th through the 13th day there were only two lines formed. On the 14th day a third line was present, i.e., line 3 in figure 8. Line 4 appeared on the 15th day and was also found on the 16th and 17th days (fig. 9). However, on the 18th day only lines 1, 2 and 3 were present and line 4 was absent (fig. 10). Lines 1, 2 and 3 remained through the 21st day and a fourth line was present at this stage, but it is not possible from the data to ascertain if this line was identical to line 4 in 15-day duodena.

The distribution of line 4 has been confirmed by repetition of experiments and also by means of the Björklund inhibition technique. When the latter technique was used results were obtained which are shown in figure 11. In

this plate six doses of 0.15 M saline extract of 18-day embryonic duodenum were put into the antiserum well prior to putting the antigens and antiserum into their respective wells. It was shown in figure 10 that 18-day duodenal antigen reacted with antiserum to form lines 1, 2 and 3. Following the inhibition procedure lines 1, 2 and 3 were absent but line 4 developed adjacent to the 15-day well (fig. 11). The presence of line 4 and also the line adjacent to the 21-day well suggests that they may be qualitatively distinct from those found in the 18-day duodenum.

The results presented in figure 12 were obtained in essentially the same manner as in figure 11 with the exception that adult chicken serum was used as the inhibiting antigen. The position of lines 1, 2 and 3 adjacent to 20- and 21-day wells is comparable to those in figure 10. This suggests that lines 1, 2 and 3 are not serum antigens. The absence of the line adjacent to the 21-day well, as illustrated in figure 11, suggests that this antigen has reactive groupings in common with serum.

Table 16 summarizes the distribution of duodenal antigens which was observed in various ages of embryos. The inhibition analyses are also summarized in this table.

From the evidence at hand, it has been demonstrated that: (1) the pattern of adult duodenal antigens found in

TABLE 16

A SUMMARY OF THE RESULTS OBTAINED WITH THE SERUM-AGAR PRECIPITIN TEST. ADULT DUODENUM ANTISERUM WAS TESTED WITH SALINE EXTRACT OF ADULT AND 11 THROUGH 21-DAY CHICK EMBRYONIC DUODENA

Antiserum	Antigen	No. Lines	Inhibition	No. Lines
Anti-Adult duodenum	11-day embryo duodenum	2		
"	12-day embryo duodenum	2		
"	13-day embryo duodenum	2		
"	14-day embryo duodenum	3		
"	15-day embryo duodenum	4	18-day embryo duodenum	1
"	16-day embryo duodenum	4		
"	17-day embryo duodenum	4		
"	18-day embryo duodenum	3	18-day embryo duodenum	0
"	19-day embryo duodenum	3		
"	20-day embryo duodenum	3	Adult Serum	3
"	21-day embryo duodenum	4	Adult Serum	3
"	21-day embryo duodenum	4	18-day embryo duodenum	1
"	Adult duodenum	9		
"	Adult Serum	3	Adult Serum	0

the developing duodenum changes during development and (2) at least three of the antigens found in the embryonic duodenum do not appear to be present in adult serum.

5. THE EFFECT OF CHORIOALLANTOIC TRANSPLANTS OF ADULT CHICKEN DUODENUM AND SPLEEN ON THE DEVELOPMENT OF ADULT DUODENAL AND SPLEEN ANTIGENS IN THE DUODENUM AND SPLEEN OF 17-DAY EMBRYOS

Antisera against saline extracts of pooled adult chicken duodenum or against adult chicken spleen were tested with saline extracts of: (1) normal 17-day embryonic chick duodenum or spleen; (2) 17-day embryonic duodenum or spleen which was stimulated by CA grafts of adult chicken duodenum; (3) 17-day embryonic duodenum or spleen which was stimulated by CA grafts of adult chicken spleen. Methods similar to those mentioned in the preceding section were used.

The antigenic pattern of the developing duodenum following CA grafts of adult chicken duodenum or spleen is shown in figure 13. In this figure the antiserum well contained anti-adult chicken duodenum and the other wells contained adult spleen stimulated 17-day embryonic duodenum (Spleen Duo), adult duodenum stimulated 17-day embryonic duodenum (Duo-Duo) and normal control 17-day embryonic duodenum (17-Duo). It may be seen that opposite wells 17-Duo, Duo-Duo and Spleen Duo, lines 1 through 4 are present.

This demonstrates the presence of an antigenic component of identical specificity in all three preparations. It will be further noted that line 5 is opposite only wells Duo-Duo and Spleen Duo. On the other hand, lines 6 and 7 are present only opposite well Duo-Duo.

Figure 14 shows an Ouchterlony plate obtained with anti-adult chicken duodenum serum and tested with antigens of adult duodenum (Adult Duo), duodenum stimulated 17-day embryonic duodenum (Duo-Duo) and untreated control 17-day embryonic duodenum. It was evident in figures 13 and 14 that grafting of adult duodenum to the CAM of 9-day chick embryos caused an increase in antigenic components from 4 to 7 in the 17-day embryonic chick duodenum. It was also observed that if adult chicken spleen was transplanted to the CAM there was one extra antigenic component found but this one was also found if duodenum was used on the CAM.

It has been shown above that the spleen of the embryo undergoes a marked increase in size subsequent to grafting adult duodenum or adult spleen to the CAM. Figure 15 shows an Ouchterlony plate obtained with anti-adult chicken spleen in the antiserum well and saline soluble antigens obtained from normal control 17-day embryonic spleen (17-day Spleen), adult spleen stimulated embryonic spleen (Spleen Spleen) and adult duodenum stimulated spleen (Duo-Spleen). Lines

1, 2 and 4 can be seen opposite well 17-day Spleen and from other plates it has been confirmed that line 4 consists of two antigenic components which are seen as lines 3 and 4 opposite the Duo-Spleen well. Only two lines can be seen opposite well Spleen Spleen but these also have been shown to each consist of two lines, therefore, it can be concluded that lines 1, 2, 3 and 4 are all present. Lines 3 and 4 are clearly seen opposite well Duo-Spleen. However, lines 1 and 2 form one line as they did opposite well Spleen Spleen. It is clear then that lines 1 through 4 are opposite each of the antigen wells and are continuous and therefore demonstrate antigenic components of identical specificity. However, because the lines are together opposite some of the wells and divided when opposite others this shows that there are quantitative differences in the various spleens. Line 5, on the other hand, is present only opposite well Duo-Spleen so that this demonstrates a clearly qualitative difference.

From the above results it can be seen that grafting of adult chicken duodenum to the CAM of 9-day chick embryos causes both quantitative and qualitative antigenic differences in the duodenum and spleen of 17-day embryos. Adult spleen, on the other hand, when grafted to the CAM produces both qualitative and quantitative changes in the antigenic picture of 17-day embryonic duodenum but in 17-day

embryonic spleen only quantitative changes were observed.

DISCUSSION

1. WEIGHT AND MORPHOLOGICAL CHANGES ON HOST ORGANS FOLLOWING CA GRAFTS

Observations presented in the preceding pages demonstrated that profound changes occurred in the chick embryo subsequent to grafting of adult chicken tissues to the CAM. The host spleen was enlarged following grafts of adult spleen, duodenum, brain and skin. The host liver was enlarged following transplants of adult liver, spleen and duodenum, while the heart was enlarged following either spleen, liver or duodenal grafts. In all cases the adult duodenum produced the most pronounced enlargement of the embryonic organs studied, i.e., spleen, liver and heart. Enlargement of the host duodenum following grafts of adult duodenum could be demonstrated only on a relative weight basis, i.e., the weight of the whole embryo decreased while the weight of the duodenum remained about the same as that of controls.

Histological studies of the enlarged spleen showed that the enlargement was primarily caused by granulopoiesis regardless of the adult organ (spleen, duodenum, skin or brain) used to stimulate it. Although the gross morphology of the host liver (bile green color) and the heart

(enlarged and pale color) was altered following CA grafts of adult chicken duodenum the microscopic anatomy was similar to that of the controls.

The hypothesis of organ-specific growth stimulation was proposed by Weiss (1947) and Ebert (1955) and has been based largely on the observations of Murphy (1916), Danchakoff (1916), Willier (1924), Weiss (1947) and Ebert (1951). These workers found that when a small fragment of adult chicken organ was transplanted to the vascular bed of an embryo (either CAM or vascular area of the blastoderm) it greatly stimulated growth of the homologous embryonic organs. Weiss and Andres (1952) observed an increase in the mitotic rate of the embryonic kidney following injections of kidney brei into the CA blood vessels and also in the mitotic rate of the kidney and liver following injections of mesonephric brei (Andres, 1955). Somewhat similar results were reported by Teir (1952) for the orbital gland in rats following intraperitoneal injections of orbital gland. Sundell and Teir (1954) also found a specific factor in the digestive tract (including the duodenum) of rats which had a mitotic-stimulating effect on the epithelium of the digestive tract. In a recent study Teir et al. (1957) presume that this mitotic-stimulating effect takes place through the nucleic acids (RNA and DNA).

Kohn (1958) has demonstrated an inhibitory factor for liver regeneration in the serum of normal rats. This supports the views of Rose (1952) who maintained that organ-specific growth control substances of homologous tissues inhibited rather than stimulated growth.

Observations made during the course of this study did not support the hypothesis of organ-specific growth stimulation. Danchakoff (1918) observed that enlargement of embryonic spleen after CA transplants of adult spleen was not confined to the spleen but extended throughout the whole mesenchyme of the host. Weiss (1953a) in re-evaluating his experiments with organ injection, found that the increase in the weight of the homologous organs could be attributed to trapping of debris and hemorrhages. Andres' (1955) observations are much less convincing when one considers the work of Wilson and Leduc (1947) and Levey (1956). The former authors question the validity of mitotic rate counts in unstandardized material while the latter author did not observe changes in the kidney, i.e., the retrogression of the mesonephros following CA grafts of 18-day embryonic mesonephros or metanephros. The observations of Saetren (1956) raise additional questions concerning the interpretation of Andres' (1955) work. He found that following the injection of macerated homologous tissue into the peritoneal cavity of rats following partial nephrectomy

or removal of a portion of the liver, there was a marked inhibition of mitoses in regenerating portions of kidney and liver.

The tracer studies of Ebert (1954) showed that when labeled tissues were placed on the CAM, homologous organs had a higher specific activity than heterologous organs. Waddington and Sirlin (1955), who used autoradiographic procedures, were unable to demonstrate organ-specific transfer from grafts to homologous tissues in early stages of amphibia. Further, Horn and House (1955) observed that when they injected tagged homogenates of liver, kidney, spleen and thymus into young mice the uptake value of the spleen was consistently higher than other organs. They suggested that the spleen was the most effective organ of the reticulo-endothelial system for removing foreign protein from the circulation. The observations of Levi-Montalcini (1952) also cast doubt on the validity of the organ-specific growth hypothesis. She found that the spleen and liver of the chick were enlarged subsequent to transplantation of mouse sarcoma 37 or 180 to the allantoic vesicle of the chick.

An alternative hypothesis for the enlargement of embryonic spleen following spleen grafts was proposed by Simonsen (1957). He believed that there was a direct transfer of cells by way of the blood vascular system from the

graft to homologous tissue where they colonized. These cells were thought to multiply and form antibodies against the host. However, this hypothesis does not explain why there is an increase in weight of heterologous tissues following duodenal, brain and skin grafts.

It was shown in the preceding paragraphs that valid objections have been raised to both organ-specific growth stimulation and direct cell transfer hypotheses for increased rates in homologous tissues following CA grafts. Observations made during the course of this study do not completely support either view. For this reason it is interesting to speculate on the factors responsible for the growth of embryonic organs following CA grafts. The observations made in this study demonstrated that the mesenchyme of the embryonic spleen exhibited a marked increase in mononucleated stem cells and granulocytes. On the other hand, enlargement of the liver following grafts of adult duodenum, liver and skin was attributed to an increase in parenchyma tissue. Both fresh and dry weights of stimulated liver were higher than those of controls. Further, the secretion of bile was accelerated as evidenced by the complete discoloration of the liver with bile. It is well established that the reticular tissue of the spleen and the reticulo-endothelial cells of the liver, i.e., stellate cells of von Küpffer, are concerned with removal of foreign

material from the blood vascular system. Horn and House (1955) maintain that following injections of various tagged tissues into young mice, the spleen consistently gave higher uptake values than other organs. These authors suggest that the spleen is the most effective organ for removing foreign protein from the circulation.

Antigenic studies made during the course of this investigation demonstrated that antigens made with stimulated spleens exhibited a different antigenic pattern from control spleens. This would indicate that grafted tissue releases materials (proteins) into the circulation which stimulate the reticulo-endothelial system of the spleen. Further, certain organs, viz., heart, liver and embryonic duodenum, probably do not contain this reticulo-endothelial stimulating material, because the spleen remained essentially normal subsequent to grafting of these organs. It also has been demonstrated in this study that the embryonic duodena contain fewer antigenic components than do adult duodena. Additional evidence in support of this view, viz., that enlargement is dependent upon an imbalance of proteins in stimulated organs, is found in the antigens made with stimulated duodena. In the latter, seven lines were present while in controls only four lines could be identified. In the spleen there was an increase in mononucleated cells, granulocytes, and multinucleated

giant cells, while in the duodenum an abundance of mononucleated cells was observed. This indicates that enlargement of organs probably was induced by a reaction to a fairly specific protein. The mechanism in this reaction is inherent in the tissues themselves, i.e., the reticuloendothelial cells have the ability to remove not only particulate matter but also dissolved materials. Lewis (1931) demonstrated that monocytes and macrophages would not only ingest foreign particulate materials but would "drink" various liquefied proteins, globulins and other complex substances. However, it is a well known fact that young animals are less reactive to foreign agents than older ones. It would seem from the antigenic differences observed in stimulated duodena and spleens that this may accelerate differentiation of the phagocytic cells following CA grafts of organs which contain these antigens.

It appears then, that removal of non-specific proteins from the blood in the embryo produces a noninfectious tpye of inflammation. The response to this stimulant is an increase in the number of specific cell types, especially granulocytes. Menkin (1945) has observed that associated with the inflammatory processes, there is concomitant hyperplasia of granulocytes in the bone marrow which may spread to other lymphatic organs such as the spleen and thymus. Thus, it would appear that enlargement of the

embryonic spleen and liver of the chick after grafting of adult tissues may be due to a noninfectious inflammatory reaction caused by an imbalance of proteins in the embryo.

Heart enlargement was probably due to compensatory reaction which was related to an anemic condition of the chicks. This anemic condition was most likely due to the fact that the spleen was almost totally given over to the production of granulocytes rather than erythrocytes. The grafts were made on the 9th day of incubation at which time Fennell (1947) observed that the definitive erythrocytes had replaced the primitive erythrocytes to become the most numerous type in peripheral blood. Thus, spleen and liver enlargement may be due in part to removal of primitive generation of blood cells and foreign substances from the blood vascular system. On the other hand, heart enlargement may be due in part to compensation.

It was also observed in the course of this study that when either normal duodenum, liver or heart were injected into the yolk sac on the 4th day of incubation they caused all the embryos to die within 72 hours. This observation is in keeping with that of Fennell (1947) who using a much more dilute inoculum of minced normal liver observed that 67% of the embryos died within 5 days after inoculation. He further observed that normal liver mince injections produced blood changes which met the requirements for

hemocytoblastosis. This reaction on the part of the host embryo also can be attribued to a reaction caused by imbalance of proteins in the embryo.

2. IMMUNOLOGICAL STUDIES

It was observed in this study that adult chicken duodenum antigens can be identified in the 11-day embryonic duodenum. However, from the 11th through the 21st day of incubation there is an increase in the number of antigens in the developing duodenum, e.g., two antigens were present in 11-, 12- and 13-day embryos; three antigens in 14-day embryos; four antigens in 15-, 16- and 17-day embryos; three antigens in 18-, 19- and 20-day embryos; and four antigens in 21-day embryos. Ebert (1952) identified an increase in the number of antigens in the development of the chick spleen. He observed three antigens in spleens of 12-day embryos and six in spleens of 18-day embryos when he used 18-day embryonic spleen antiserum.

The appearance of an antigen on the 15th day and its disappearance on the 18th day is rather striking. A somewhat similar situation has been reported by Telfer and Williams (1953) in the Cecropia silk worm. They identified five pupal blood antigens present throughout metamorphosis with a sixth appearing at the fifth instar. This persisted during the pupal stage and disappeared in the adult. In the case reported in this study the antigen

observed on the 15th day was also found in the adult because antiserum was prepared against adult duodenum. It must be kept in mind that at the present time it is not possible to prove the complete absence of an antigen on the basis of its absence in agar plates.

Several explanations for the appearance and disappearance of an antigen during the course of development have been advanced. Tyler (1957) suggested that many of the large molecular substances of an organism may have similar determinant groups and therefore may react with the same antiserum. Observations made during the course of this study lead one to believe that this view does not adequately explain the disappearance of antigens in the course of development.

During morphogenesis there may be a structural change in antigenic macromolecules. Fox (1958) proposed that an antigen may be transformed from a complete antigen into an incomplete antigen. An incomplete antigen is one which has the specificity for antibody formation but it will not react in the precipitin test. A somewhat different change in antigenic structure could occur, i.e., a change from a globular to a fibrous protein. Mazia and Dan (1952) observed that during spindle fiber formation there was a change from globular to fibrous protein. Heidelberger (1956) suggests that antigenic specificity may be due to

multiple reactive areas on the surface of antigens. He believes that changes in specificity of native proteins can be accounted for by the unfolding of polypeptide chains which leads to their separation from adjoining chains and as a consequence the specificity of the antigen is altered.

If Heidelberger's concept correctly explains antigen specificity it is reasonable to assume that during the course of development of an antigen its polypeptides may undergo foldings and separations. Under these conditions the reactive surface of the molecule is altered and cannot be detected by immunological methods. On the basis of the evidence on hand and the wide divergence of views of antigen reactivity, a final explanation cannot be given why line 4 appears in 15-day duodena and disappears in 18-day duodena.

It has been further observed in the course of this study that transplantation of adult chicken organs (duodenum and spleen) to the CAM caused changes in the antigenic pattern of the duodenum and spleen. This change was most pronounced following grafting of adult duodenum. Adult duodenal grafts increased the number of antigens from four to seven in the 17-day embryonic duodenum and from four to five in the spleen. The grafting of adult spleen altered the antigenic pattern of the 17-day duodenum (from four

to five) but was without effect in the spleen. It must be kept in mind, however, that even these changes in the antigenic picture are the end products of differentiation and not the causal factors.

Ebert (1955) maintained that growth of host tissues following homologous grafts was due to a "building block" rather than a "template" or catalytic mechanism (Weiss, 1947). The findings in this investigation tend to support this "building block" hypothesis, i.e., selective incorporation of tissue-specific antigens from grafts into homologous host tissues. However, this hypothesis does not adequately explain the generalized effects, i.e., growth of heterologous organs. There is evidence that a growing organ will remove from the blood vascular system metabolites which play an important part in growth. Kato and Moog (1958) found that after injection of disodium phenylphosphate into the chick on the 14th day of incubation there was an increased alkaline phosphatase activity in the duodenum, liver, mesonephros and metanephros on the 17th day of incubation.

It was shown in this study that the spleen, duodenum, skin, brain and liver stimulated growth of embryonic organs. It is interesting to speculate on the factors responsible for the growth of these organs. If it is assumed that a possible increase in substrate concentration in the embryonic

circulatory system could ensue following grafting of adult tissues, then growth may be attributed to specificity of substrate for certain organs. Under certain of these conditions these enzyme systems would be accelerated and growth would be enhanced.

SUMMARY

1. It was observed in this study that following CA grafts of adult chicken duodenum there was a marked decrease in the absolute weight of the host, a marked increase in the weight of the spleen, liver and heart and a relative weight increase in the duodenum. Further, following grafts of adult skin and brain the spleen and liver were significantly heavier. Following liver grafts the liver and heart showed a significant increase in weight. Adult chicken spleen grafts caused a marked increase in the weight of the spleen and heart. Further, it was observed that regardless of what tissue was used for grafting, nine days later the morphological integrity of the graft was destroyed and the area was replaced by a myeloid metaplastic center. On the other hand, embryonic duodena retained their integrity and continued to differentiate.

2. Grafting of adult duodenum caused acceleration of tissue differentiation of the host duodenum. Following grafting of duodenum, spleen and skin the host spleen exhibited a marked increase in granuloblasts and granulocytes. The heart and liver following grafts were essenially like those in control embryos.

3. Observations made following treatment of the adult duodenum prior to grafting with either 95% alcohol for 24

hours at -20° C, lyophilization, heating at 80° C for 20 minutes or treatment with 1:10,000 merthiolate in 0.15M sodium chloride for 30 minutes, showed that the grafts were inactivated and the host was not effected.

4. When duodena of 15-, 16-, 17-, 18- and 20-day embryos were grafted to the CAM no changes in the weight of host spleen, liver or heart were found. Following 20-day duodenal grafts the weight of the duodenum was significantly decreased.

5. All embryos receiving grossly normal adult duodenum, liver and heart supernatant injections into the yolk sac at four days of incubation died within 72 hours after inoculation.

6. The polysaccharides in the connective tissue and goblet cells of the duodenum following CA grafts of adult duodenum, differentiated at least 24 hours earlier than in control chicks.

7. The development of adult duodenal antigens in the embryonic duodenum was studied by means of the Ouchterlony agar diffusion technique. In 11-, 12- and 13-day embryonic duodena there were two antigens; by the 14th day three antigens; in the 15-, 16- and 17-day duodena four antigens; while in 18-, 19- and 20-day duodena there were only three antigens with a fourth antigen again present in the 21-day duodenum. The disappearance of the fourth antigen

found in 15-, 16- and 17-day duodena was attributed to a change in molecular configuration in the process of differentiation.

8. Following grafts of adult duodenum there was an increase of three antigens observed in the host duodenum and an increase of one antigen in the host spleen. When adult spleen was grafted there were no extra antigens in the host spleen but there was one extra antigen in the duodenum.

9. The results are discussed in the light of the organ-specific growth stimulation hypothesis and this hypothesis is rejected. It has instead been postulated that the adult grafts may provide substrate for enzyme systems in host organs and this upsets the balance and causes the more generalized results reported in this study.

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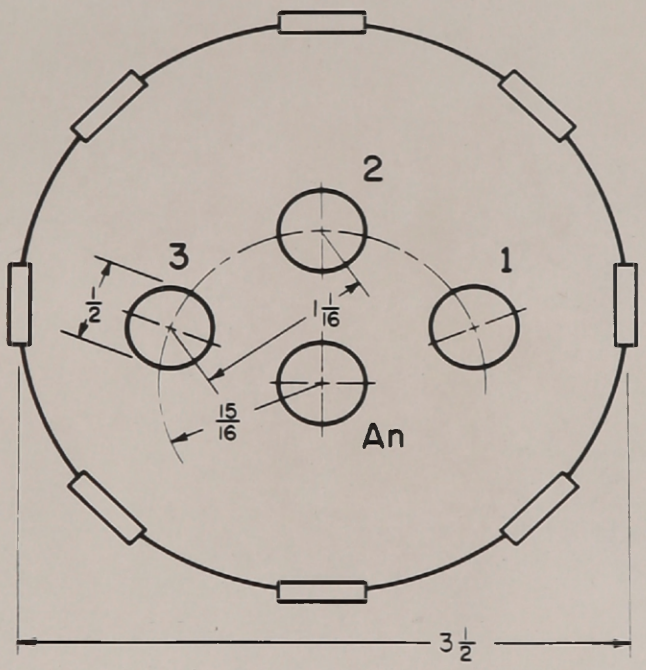
PLATE I

Fig. 1. Geometry of an Ouchterlony plate.

An. Antiserum well;
1. Antigen well;
2. Antigen well; and
3. Antigen well.



1

PLATE II

Fig. 2. Cross section through the duodenum of a control 18-day embryo.

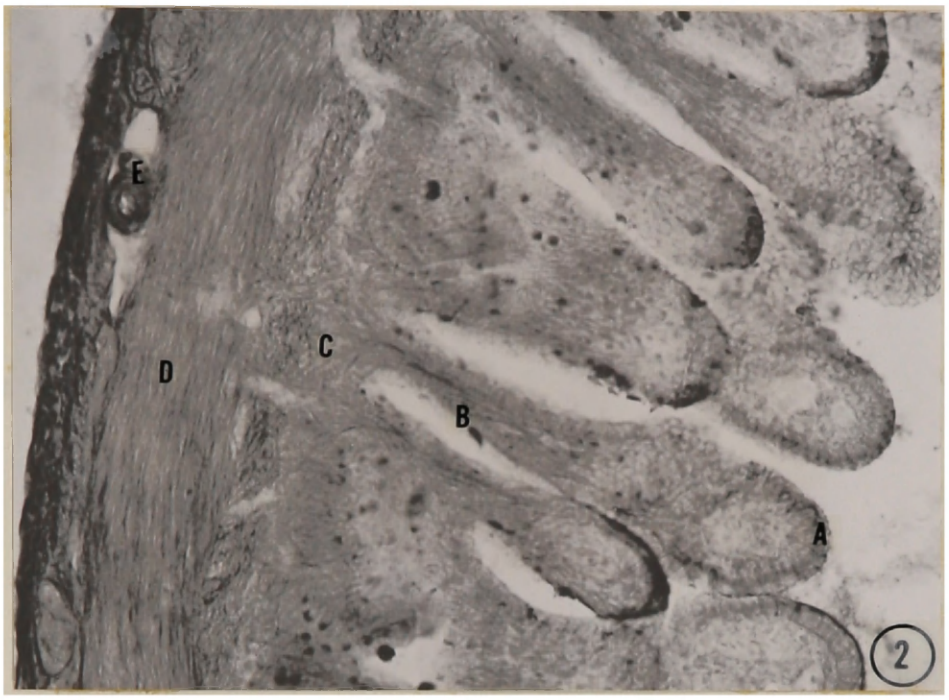
A. Epithelium;
B. Goblet cell;
C. Lamina propria;
D. Tunica muscularis;
E. Blood vessel.

Fig. 3. Cross section through the duodenum of an 18-day embryo following adult chicken duodenal grafts.

A. Epithelium;
B. Goblet cell;
C. Lamina propria;
D. Tunica muscularis;
E. Blood vessel;
F. Lymphocytes.

Micrometer scale insert:1 space= 0.01 mm.

All sections were stained with Himes and Moriber triple stain.


E
D
C
B
A
2

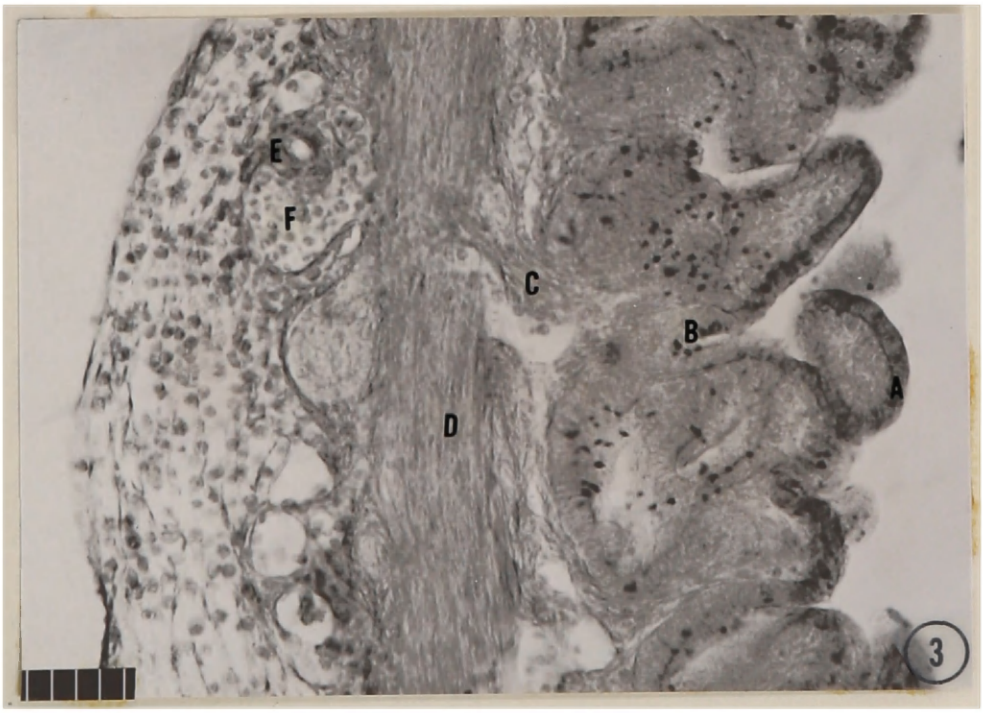

E
F
C
B
A
D
3

PLATE III

Fig. 4. Cross section through a spleen of a control 18-day embryo.

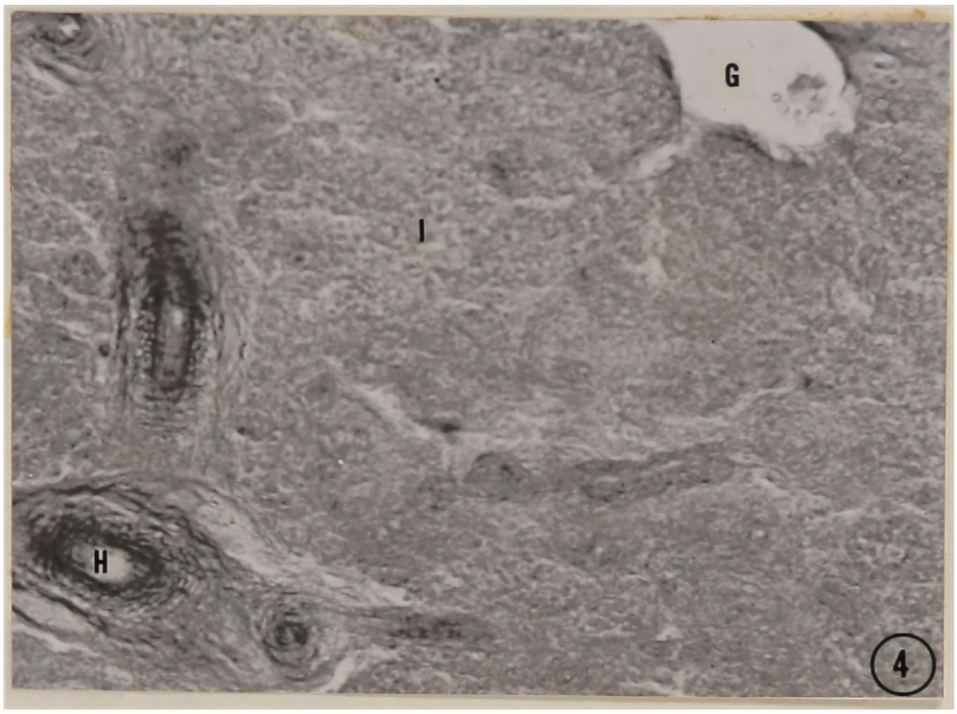
G. Vein;
H. Artery;
I. Reticular structure

Fig. 5. Cross section through a spleen of an 18-day embryo following adult duodenal grafts.

G. Vein;
H. Artery;
J. Nodule;
K. Multinucleated giant cells;
L. Lymphoid hemocytoblast.

Micrometer scale insert; 1 space = 0.01 mm.

All sections were stained with Himes and Moriber triple stain.


G
I
H
4

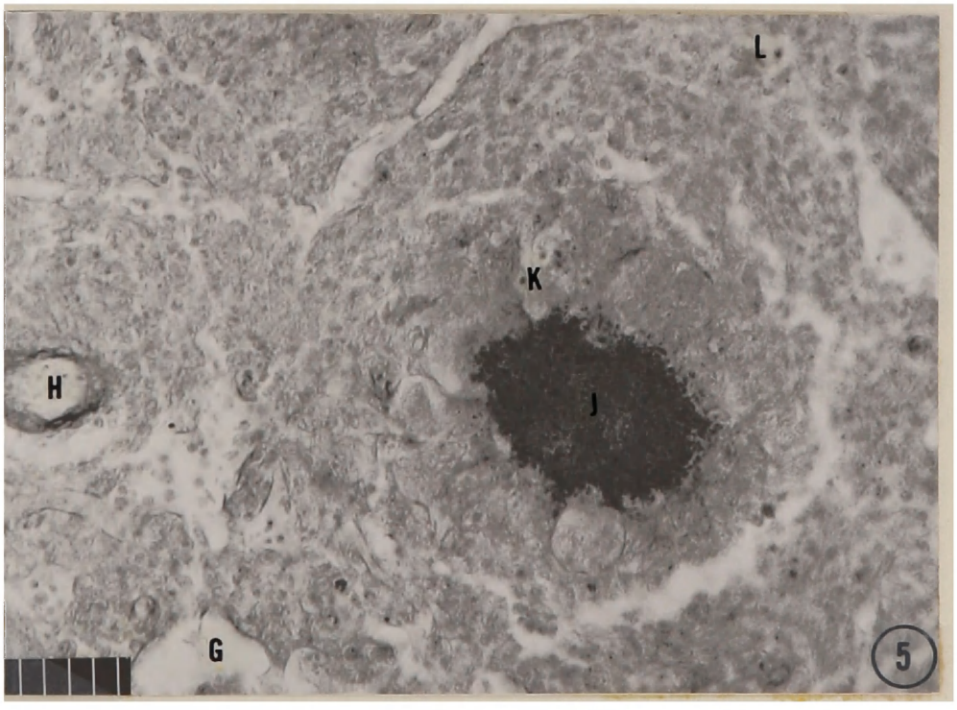

L
K
H
J
G
5

PLATE IV

Fig. 6. Cross section through an adult duodenal graft on the 18th day of incubation.

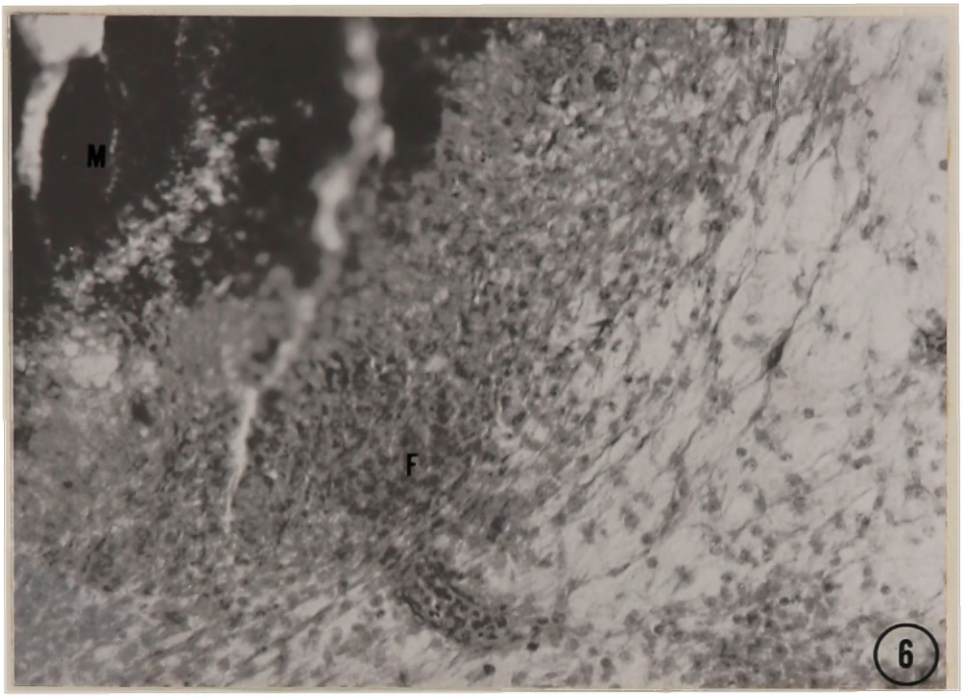
F. Lymphocytes and granulocytes;
M. Foci of degenerating adult duodenal tissue.

Fig. 7. Cross section through a 15-day embryonic duodenal graft on the 18th day of incubation.

A. Epithelium;
B. Goblet cell;
C. Lamina propria;
D. Tunica muscularis;
E. Blood vessel;
N. Chorioallantoic membrane.

Micrometer scale insert: 1 space = 0.01 mm.

All sections were stained with Himes and Moriber triple stain.


M
F
6

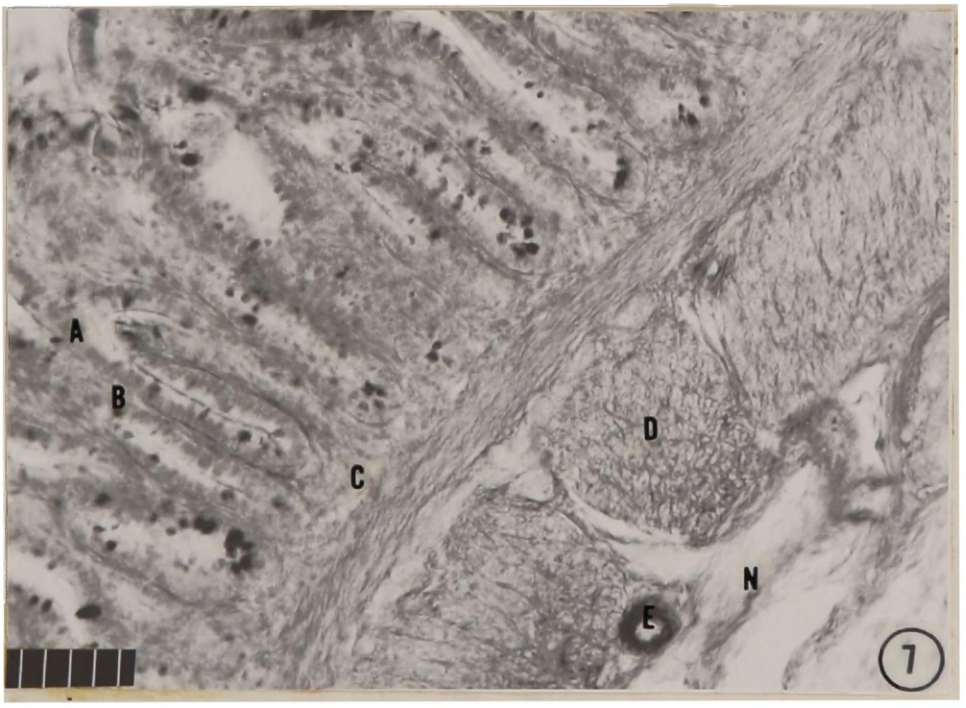

A
B
C
D
N
E
7

PLATE V

Fig. 8. Photograph and diagram of an Ouchterlony plate.

An. Anti-adult chicken duodenum;
12-Duo. Antigen of 12-day embryonic duodenum;
13-Duo. Antigen of 13-day embryonic duodenum;
14-Duo. Antigen of 14-day embryonic duodenum.


13
14
12
S-1-D
1-B-9

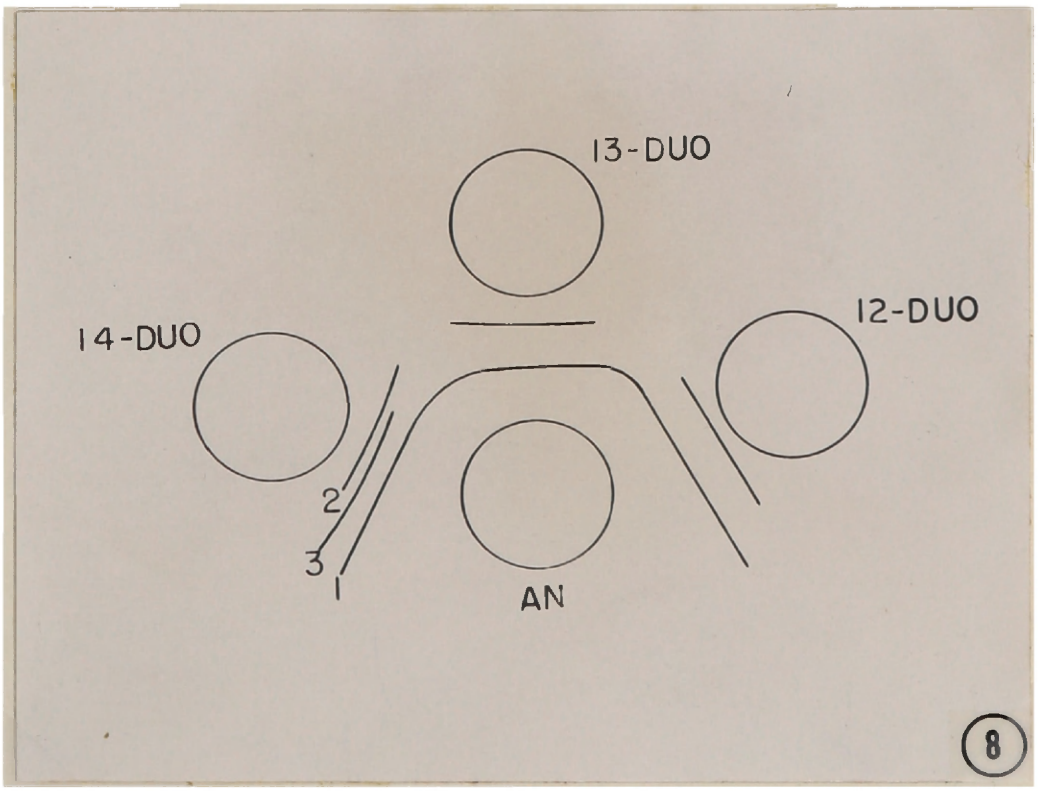
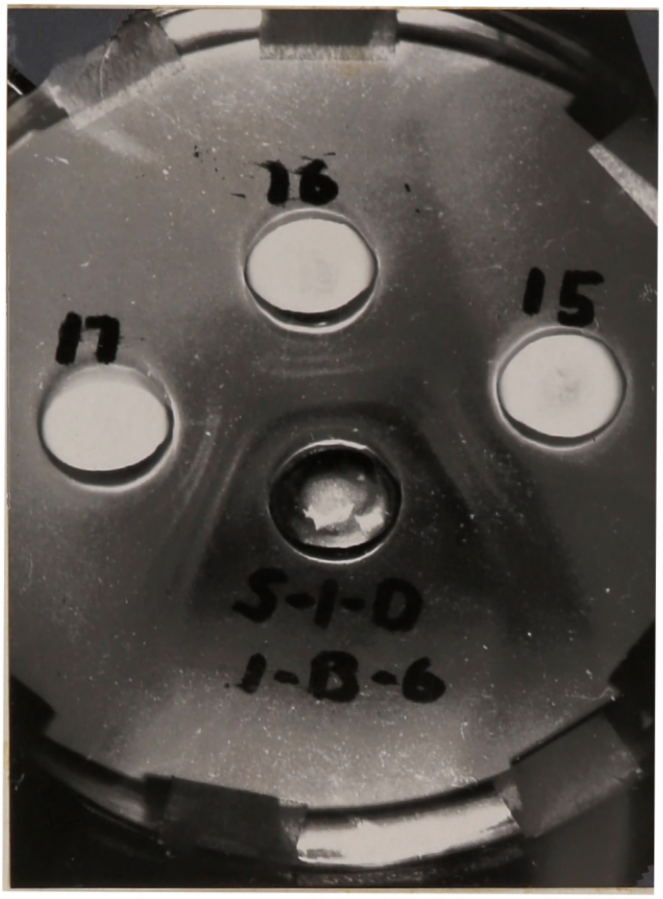

13-DUO
14-DUO
12-DUO
2
3
1
AN
8

PLATE VI

Fig. 9. Photograph and diagram of an Ouchterlony plate.

An. Anti-adult chicken duodenum;
15-Duo. Antigen of 15-day embryonic duodenum;
16-Duo. Antigen of 16-day embryonic duodenum;
17-Duo. Antigen of 17-day embryonic duodenum.


16
15
17
S-1-D
1-B-6

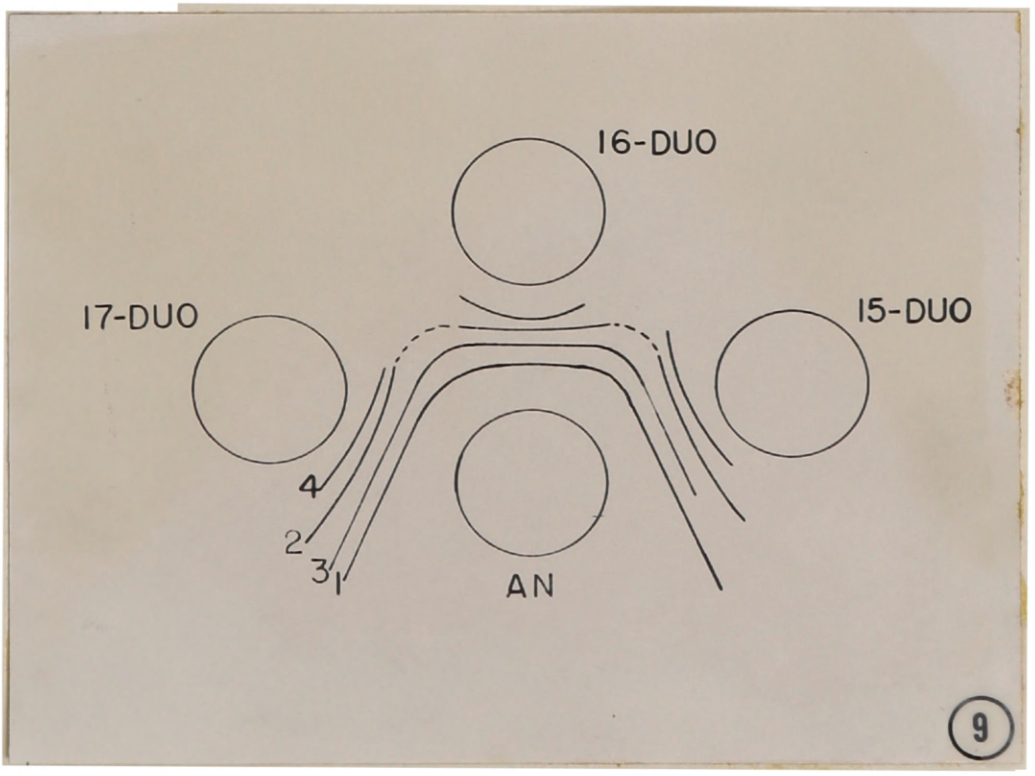

16-DUO
17-DUO
15-DUO
4
2
3
1
AN
9

PLATE VII

Fig. 10. Photograph and diagram of an Ouchterlony plate.

An. Anti-adult chicken duodenum;
18-Duo. Antigen of 18-day embryonic duodenum;
19-Duo. Antigen of 19-day embryonic duodenum;
20-Duo. Antigen of 20-day embryonic duodenum.


19
20
18

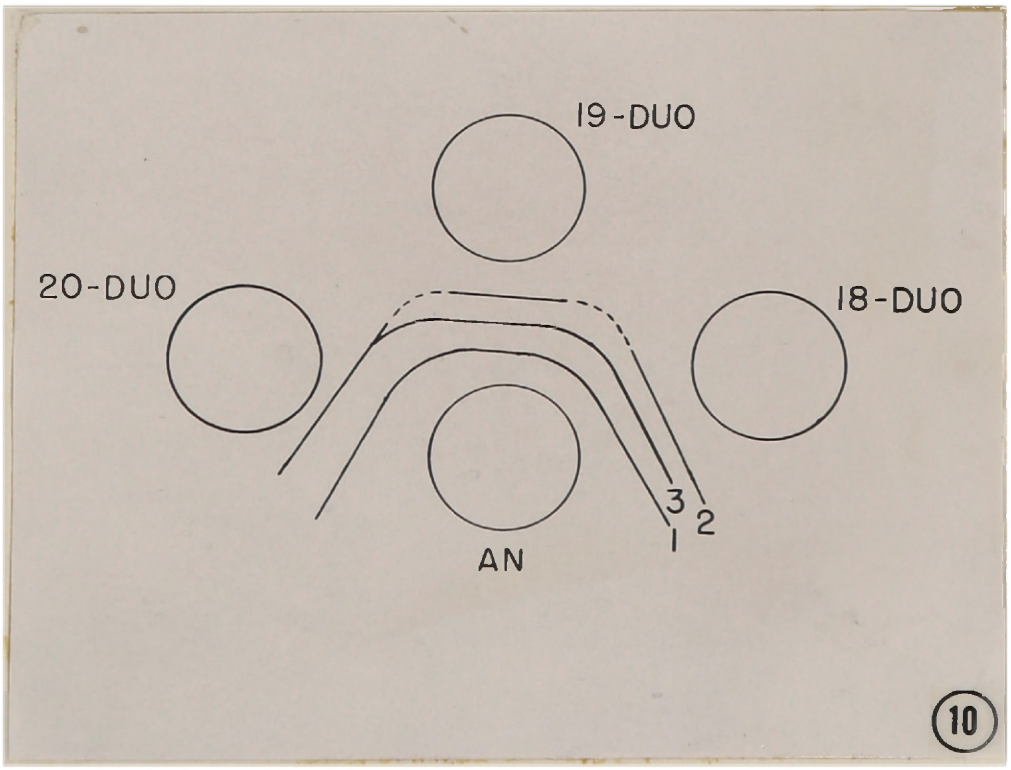
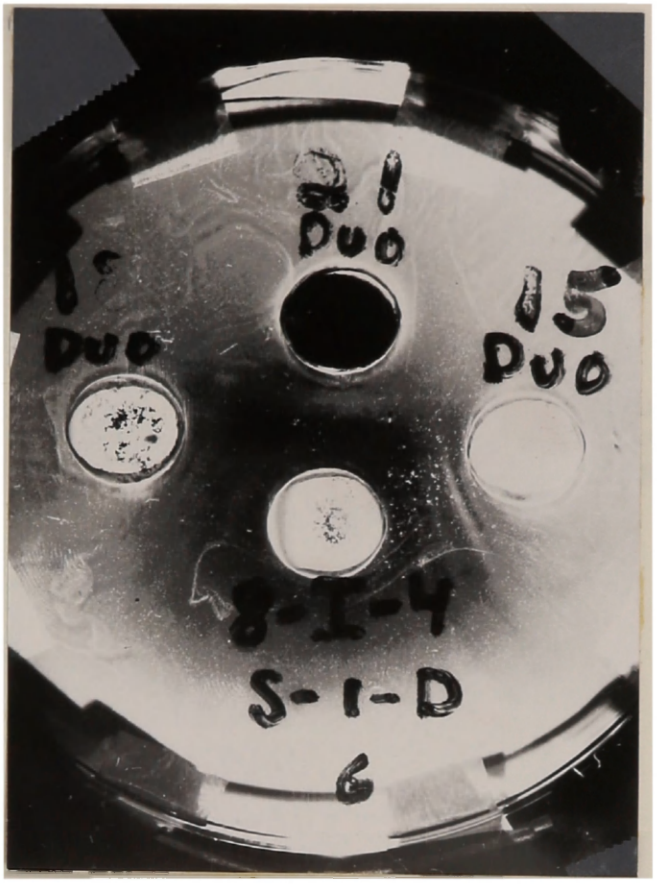

19-DUO
20-DUO
18-DUO
3
2
1
AN
10

PLATE VIII

Fig. 11. Photograph and diagram of an Ouchterlony-Björklund inhibition plate.

An. Six doses of 18-day embryonic duodenum antigen prior to anti-adult duodenum;
15-Duo. Antigen of 15-day embryonic duodenum;
21-Duo. Antigen of 21-day embryonic duodenum;
18-Duo. Antigen of 18-day embryonic duodenum.


DUO
DUO
15
DUO
S-1-D

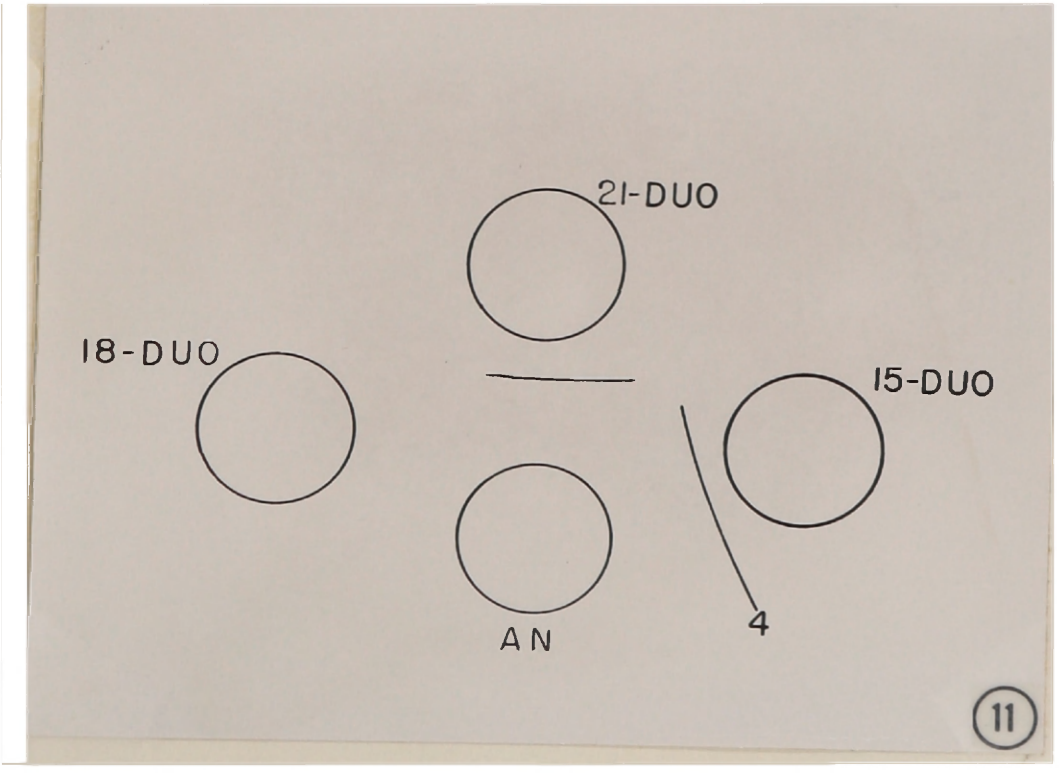

21-DUO
18-DUO
15-DUO
AN
4
11

PLATE IX

Fig. 12. Photograph and diagram of an Ouchterlony-Björklund inhibition plate.

An. Four doses of adult chicken serum prior to anti-adult duodenum;
20-Duo. Antigen of 20-day embryonic duodenum;
21-Duo. Antigen of 21-day embryonic duodenum;
Serum. Blood serum of adult chickens.


21
SE
20
S-1-D
9-I-1

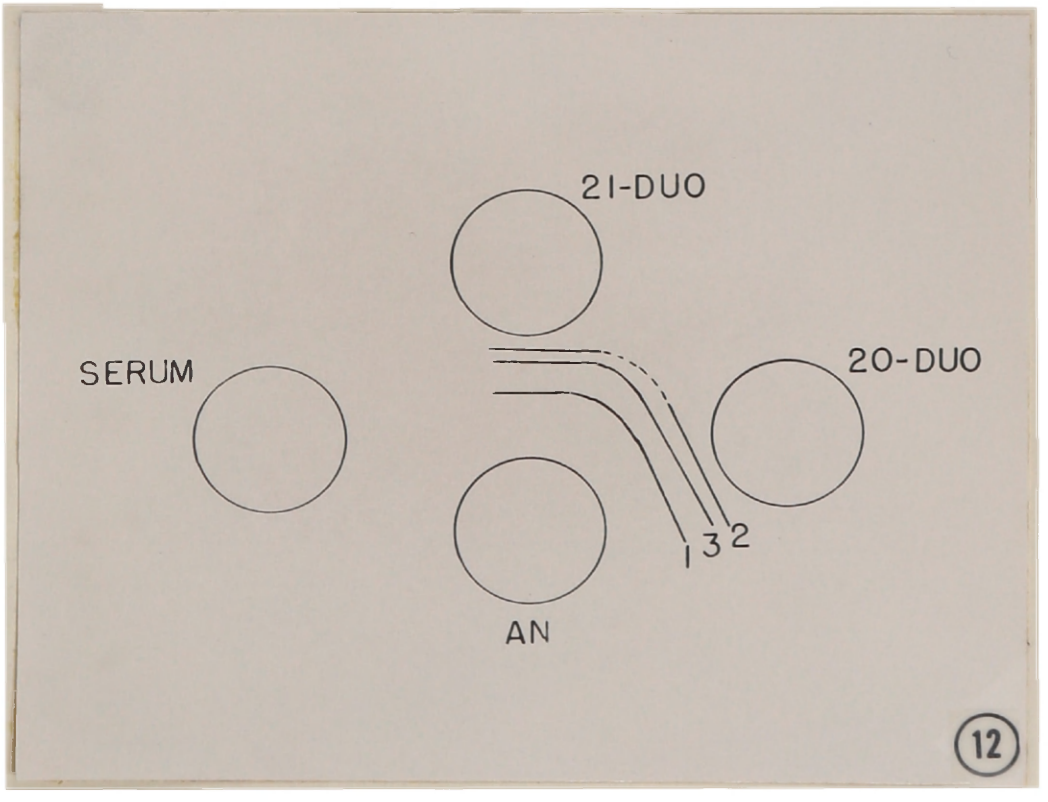
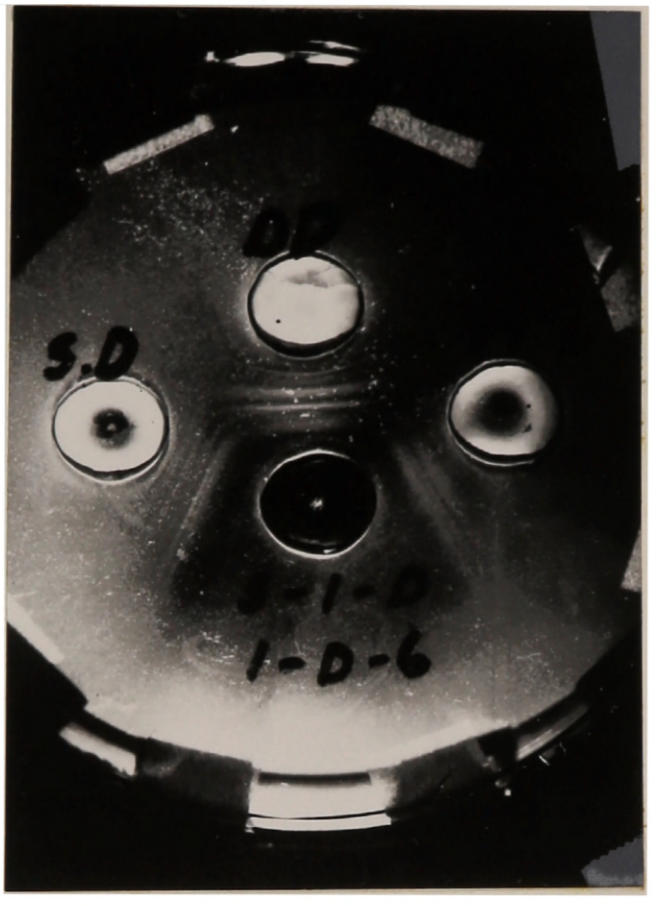

21-DUO
SERUM
20-DUO
1 3 2
AN
12

PLATE X

Fig. 13. Photograph and diagram of an Ouchterlony plate.

An. Anti-adult duodenum;
17-Duo. Antigen of control 17-day embryonic duodenum;
Duo-Duo. Antigen of 17-day embryonic duodenum following CA grafts of adult duodenum;
Spleen Duo. Antigen of 17-day embryonic duodenum following CA grafts of adult spleen.


S.D
1-D-6

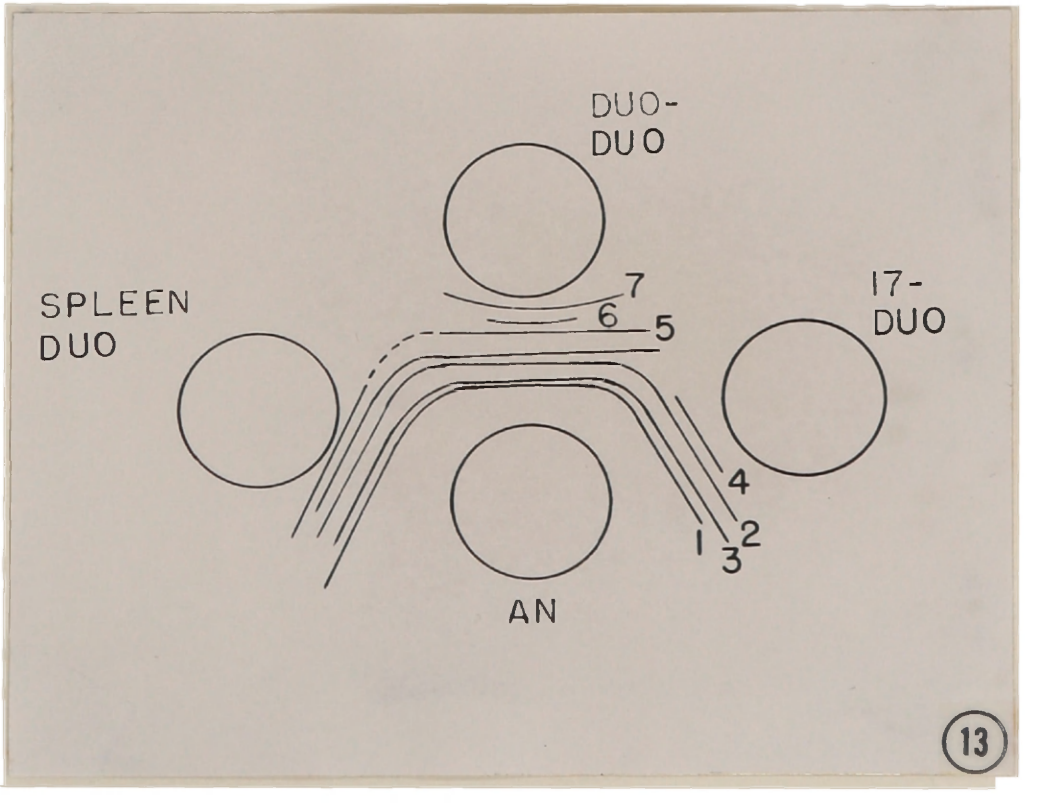
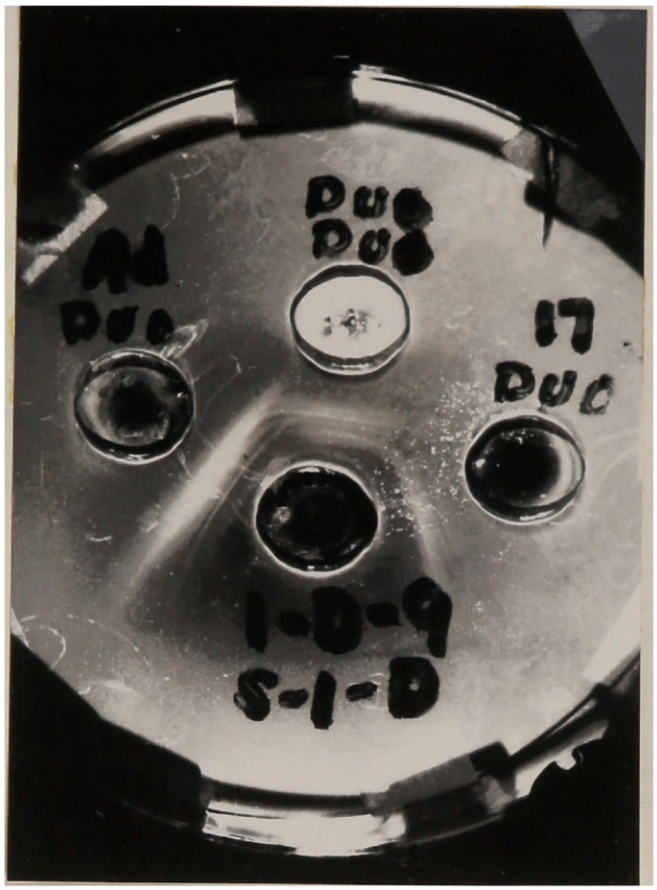

DUO-
DUO
SPLEEN
DUO
17-
DUO
7
6
5
4
1
3
2
AN
13

PLATE XI

Fig. 14. Photograph and diagram of an Ouchterlony plate.

An. Anti-adult chicken duodenum;
17-Duo. Antigen of control 17-day embryonic duodenum;
Duo-Duo. Antigen of 17-day embryonic duodenum following CA grafts of adult duodenum;
Adult Duo. Antigen of pooled adult duodena.


DUO
DUO
AN
DUO
17
DUO
1-D-9
S-1-D

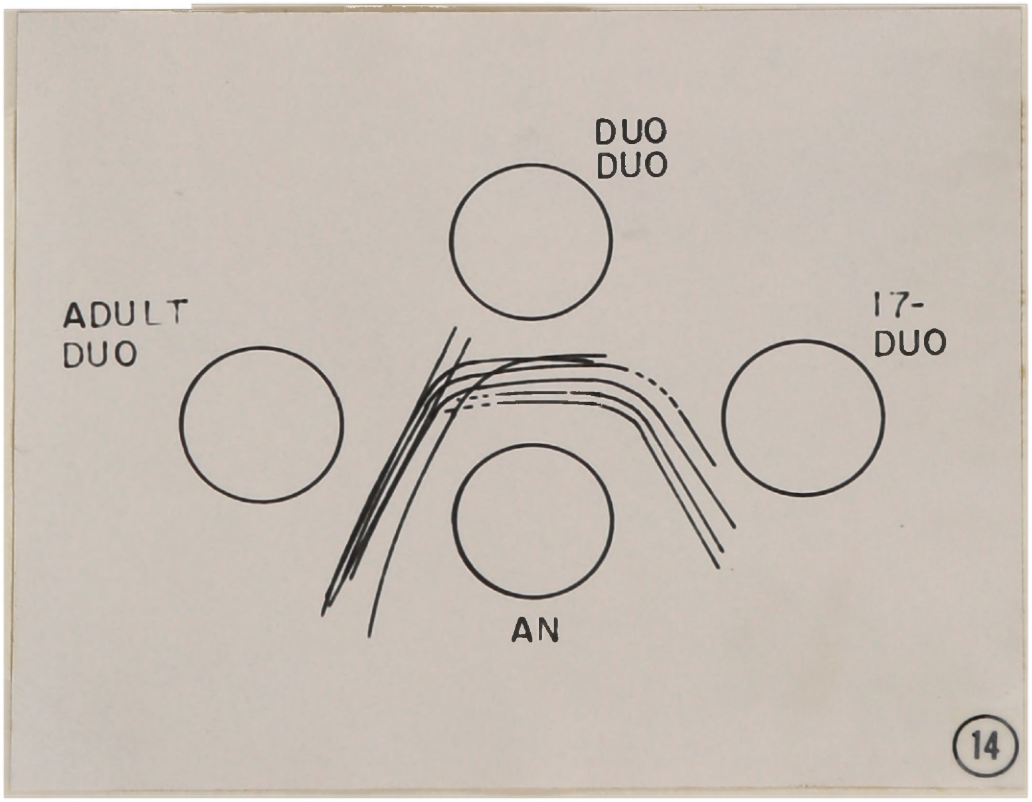
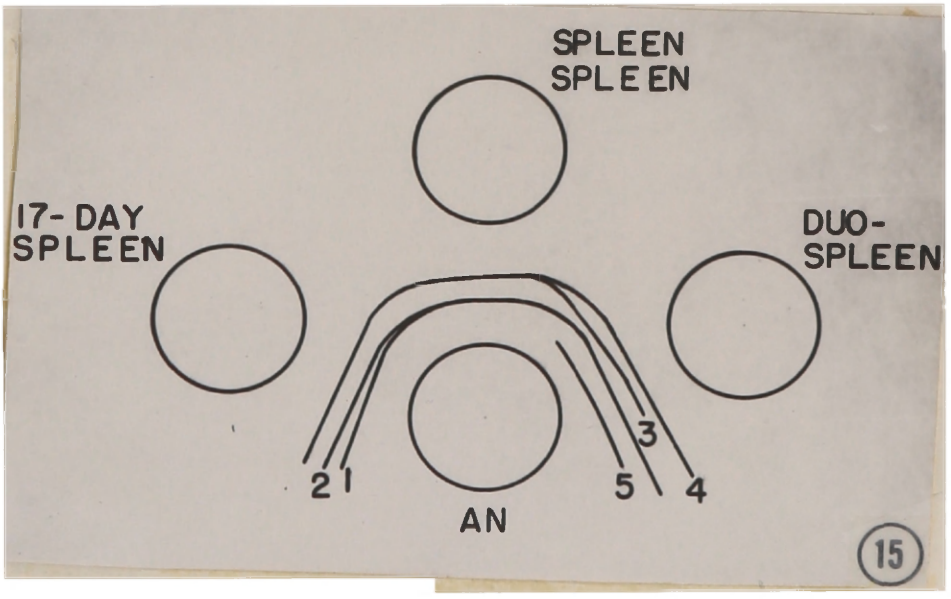

DUO
DUO
ADULT
DUO
17-
DUO
AN
14

PLATE XII

Fig. 15. Photograph and diagram of an Ouchterlony plate.

An. Anti-adult chicken spleen;
Duo-Spleen. Antigen of 17-day embryonic spleen following CA grafts of adult duodenum;
Spleen Spleen. Antigen of 17-day embryonic spleen following CA grafts of adult spleen;
17-Day Spleen. Antigen of control 17-day embryonic spleen.


S-Sp
D-Sp
S-1-S
1-D-2


SPLEEN
SPLEEN
17-DAY
SPLEEN
DUO-
SPLEEN
2
1
3
5
4
AN
15