

## TRANSPLANTATION AND INDIVIDUALITY DIFFERENTIALS IN INBRED FAMILIES OF GUINEA-PIGS \*

LEO LOEB AND SEWALL WRIGHT

*(From the Department of Pathology, Washington University School of Medicine,  
St. Louis, Mo., and U. S. Department of Agriculture, Washington, D. C.)*

In a series of papers one of us analyzed the reactions of the host against auto, syngenesio, homoio and heterotransplants.<sup>1</sup> Definite relations were found between the intensity of the lymphocytic and connective tissue reactions and the genetic relationship between host and donor. The similarity or differences between the individuality differentials of host and transplant largely determined the effects of the transplantation. These relations between individuality differentials decided whether the substances given off by the transplant affected the host as normal auto substances or as toxins of various intensities (syngenesio, homoio, heterotoxins). In the case of auto-transplantation, there exists an identity of individuality differentials. The intensity of the reactions appearing after transplantation furnished a quantitative measure of the similarity or difference between individuality differentials. It was to be assumed that through long-continued inbreeding the individuality differentials among the members of the inbred family would gradually become more and more alike. In addition to these primary factors affecting the individuality differential there were active secondary factors of a non-genetic character.

Under these conditions it was of interest to extend these experiments to inbred strains, and this paper deals with the results obtained in the exchange of tissues in families of guinea-pigs which have been inbred in the United States Department of Agriculture since 1906. Five different families have thus been developed through continued sister and brother matings (2, 13, 32, 35 and 39). However, the degree of homogeneity in the various families differs.<sup>2</sup> In addition, control experiments were made in which tissues were exchanged with members of a B group. In this group, originally derived from the same stock as the inbred families, matings as close as those between second cousins have been avoided. These latter

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experiments approached therefore homoiotransplantations.\* Each guinea-pig was checked by a number assigned serially and given here in brackets. Each inbred mating is designated by three figures. The first is the family number (2, 13, 32, 35 and 39); the second is the number of generations of brother-sister mating since the foundation (O) mating of the family; while the third is arbitrary. Experiments 2N, Y and 35I involve only families 2, 13 and 35 respectively but are not brother-sister matings. Experiment CO is composed of crosses between inbred families.

#### I. TRANSPLANTATIONS IN THE SAME FAMILY, HOST AND DONOR NOT BEING NEARLY RELATED

SERIES A. *Experiments in which the examination took place within two months following transplantation.* Weight of guinea-pigs in most cases varying between 200 and 500 gm.

1. From guinea-pig 32-17-10 (15611) to guinea-pig 32-19-8 (321), 40 days. Both thyroid and cartilage transplants are like perfect autotransplants, as far as structure and lack of increase in lymphocytes and connective tissue are concerned. Grade 6.

2. From 2-17-10 (15959) to 2-17-5 (15694) 50 days. Thyroid, parathyroid, and cartilage autotransplants. Grade 6.

3. Y-4  $\left\{ \begin{array}{l} 13-20-2 \\ 13-19-5 \end{array} \right\}$  (16039) to Y-7  $\left\{ \begin{array}{l} 13-20-2 \\ 13-19-6 \end{array} \right\}$  (16029) 40 days.

Syngenesio-reaction. Grade 5. Thyroid shows auto-structure, but there is much lymphocytic infiltration in certain areas of the center. From here lymphocytes penetrate between acini toward periphery and surround some acini. Around a vessel traversing the ring of acini are lymphocytes. In other places the center is free from lymphocytes. Cartilage transplant with areolar tissue is well preserved; but there is some increase of connective tissue around the cartilage and an incomplete mantle of lymphocytes.

4. From 32-17-11 (15575) to 32-17-8 (15634) 40 days. Thyroid autotransplant. Grade 6.

5. From 13-19-9 (15649) to 13-20-5 (15563) 35 days. Syngenesio-reaction. Grade 5. Thyroid with auto-structure; parathyroid well preserved, but intense lymphocytic infiltration around and in parathyroid, and in places in the periphery of the thyroid. In various

\* These figures have been mentioned in a previous paper.

places small parts of thyroid have been destroyed by lymphocytes which accumulate around vessels. Normal cartilage surrounded by areolar and fat tissue, with only a slight increase in connective tissue and lymphocytes.

6. From 13-20-5 (15563) to 13-20-13 (15782) 31 *days*. Syngenesio-reaction. Grade 5. Lobes of thyroid were transplanted (parts of the transplants were used for retransplantation). Structure of autotransplants, but in center there is moderate, although distinct, lymphocytic infiltration; lymphocytes penetrate also between acini and surround and destroy some of them. Moderate increase of connective tissue between acini.

7. From 32-18-9 (20865) to 32-19-9 (16900) 38 *days*. Resembles autotransplant. Grade 6.

8. From 2-18-4 (15693) to 2-16-16 (15590) 49 *days*. Syngenesio-reaction. Grade 5. Well preserved thyroid. Considerable lymphocytic infiltration penetrating a little toward periphery and also into parathyroid. Slight connective tissue increase around cartilage. Slight increase of lymphocytes in areolar tissue.

Eight experiments constitute this series. In four cases an auto-reaction was obtained; in four cases a syngenesio-reaction. In the latter cases the transplant first began to develop as an autotransplant, but later, lymphocytes began to invade the transplant and even connective tissue penetrated between some of the acini. We have, therefore, in these cases to deal with syngenesio-reaction. Auto-reactions were found 38, 40 and 50 days after transplantation, syngenesio-reactions 30-50 days after transplantation. Auto-reactions were obtained in families 32 and 2, syngenesio-reaction in family 13, and in one case in family 2. There can be no doubt that a difference existed in the constitution of the individuality differentials in the members of families 13 and 2 which showed syngenesio-reaction. In the two cases in family 32 and in one case in family 2 the reactions did not indicate a difference in individuality differentials, but this does not necessarily exclude the possibility that such a discrepancy would not have been revealed if we had extended the experiments over a still longer period. On the other hand we can state definitely that the members of the same inbred family are more nearly akin to each other as far as the constitution of their individuality differential is concerned, than in ordinary guinea-pig families where brothers are related to each other.

**SERIES B.** *In a second similar series the examination took place at a still later date in the majority of cases. In these experiments the weight of the animals was generally greater, in accordance with the greater age of these guinea-pigs.*

1. From 13-20-5 (15563) to 13-20-13 (15782) *5 months, 12 days.* Transplanted thyroid was not found.

2. From Y-7  $\left\{ \begin{array}{l} 13-20-2 \\ 13-19-6 \end{array} \right\}$  (16029) to 13-20-13 (15782) *5 months, 12 days.* In this case there is a decided syngenesio-reaction. A part of the transplanted thyroid was preserved and the acini contain well formed colloid. But there is much lymphocytic infiltration. The lymphocytes penetrate everywhere between the acini and even into the epithelium and destroy it. There is also some increase of fibrillar connective tissue around some acini. Lymph vessels are filled with lymphocytes. Large masses of these cells surround and encompass acini which have lost their colloid. Grade 4.

3. From 2N-27 (18040) to 2-17-14 (18450) *102 days.* Thyroid and cartilage were perfect autotransplants. Grade 6.

4. From 13-19-15 (18317) to 13-21-7 (18493) *102 days.* Thyroid, parathyroid and cartilage with surrounding tissue resemble autotransplants, except that in one place in the cartilage transplant there was a slight collection of lymphocytes around a vessel; this latter condition may still come within the range of autotransplant. Grade 6 or 5.75.

5. From 2-16-16 (15590) to 2N-26 (15673) *104 days.* Auto-reactions in thyroid and cartilage transplant. Grade 6.

6. From 32-19-8 (15634) and 32-19-8 (15633) to 32-19-9 (16900) *37 days.* Two thyroids, and parathyroids transplanted. Auto-reaction, except that in one place in the center there is a considerable lymphocytic infiltration. Grade 5.60.

The six experiments of this series confirm and extend the conclusions of the first series. Experiment 1 is uncertain as to the interpretation of the results, no thyroid transplant having been found after 5 months, 12 days. It is possible that the transplant has been destroyed after such a long period, especially considering the fact that transplantation was carried out in family 13, in which there is apparently a greater tendency on the part of the host to react against the transplant from another individual of the same family. A marked reaction was obtained in family 13 in the second case follow-

ing a transplantation extending over a long period of time. In the fourth case, on the other hand, we find after almost three and a half months a condition approaching that found in autotransplants. It is, of course, to be assumed that in certain combinations of animals of this family the individuality differentials may happen to be identical or almost identical. In two experiments in family 2 the results indicated an identity, or a condition almost approaching identity, of the individuality differentials. In one experiment in family 32, extending over 37 days, the result at present is similar to that obtained in autotransplantation, but there is some indication that with an extension of the experiment, the differences between the individuality differentials would come out definitely. These experiments then demonstrate the lack of identity of individuality differentials in family 13 and probably also in family 32. The pedigree analysis indicates that the large number of reactions not auto in family 13 is probably due to the large number of experiments in which host and donor had less than 10 generations of common inbreeding. At the same time these experiments prove that the similarity of individuality differentials is still greater than appeared in the first experiment, inasmuch as even after relatively long periods of time the conditions obtained may still correspond to those found in autotransplantation. And even where, as in some cases in family 13, a definite discrepancy between the individuality differentials has been found, the latter are still much more closely related than the average of the individuality differentials of brothers in non-inbred families of guinea-pigs.

These experiments demonstrate anew the great sensitiveness of the lymphocytic reaction which may appear at a very late stage of transplantation and then exert a destructive effect in case the syngenesio toxins are very weak. There may be also associated with this lymphocytic reaction a secondary slight proliferation of the connective tissue in the transplant.

SERIES C. *Transplantation of tissues from a hybrid between two inbred species to another hybrid of similar constitution.*

- I. From C-O-297  $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$  (17093) (320 gm.) to  
 C-O-234  $\left\{ \begin{array}{l} 2-13-7 \\ 35-16-20 \end{array} \right\}$  (15880) (632-682 gm.)

31 days. Thyroid with auto-structure; in one place in center there is a considerable lymphocytic infiltration; the lymphocytes infiltrate

also parathyroid and surround some acini of thyroid. Cartilage similar to autotransplant; but there is in places in the surrounding areolar and fat tissue a slight increase in connective tissue with some lymphocytes around vessels. Near such places in fat tissue are found some giant and epithelioid cells; elsewhere fat tissue is free from the latter structures. Grade 5.

2. From C-O-285  $\left\{ \begin{array}{l} 35-21-2 \\ 32-15-15 \end{array} \right\}$  (15942) to  
 C-O-240  $\left\{ \begin{array}{l} 32-16-9 \\ 35-16-11 \end{array} \right\}$  (15943)

35 days. Thyroid and cartilage resemble autotransplant. Grade 6. The number of experiments in this series is too small to allow of any definite conclusion.

SERIES D. *Successive transplantation in the same family.* In a few cases, pieces were transplanted to an animal of the same strain, and after these pieces had been in the first host for some time, they were removed and retransplanted into a second host belonging likewise to the same strain. After a certain period these retransplanted pieces were taken out and examined microscopically.

1. First transplantation. From 13-20-5 (15563) and from

$$Y-7 \left\{ \begin{array}{l} 13-20-2 \\ 13-19-6 \end{array} \right\} (16029) \text{ to } 13-20-13 (15782) \text{ 31 days.}$$

Second transplantation. 13-20-13 (15782) to 13-18-9 (16174) 23 days. Thyroid shows structure of autotransplant: a ring of acini with a small amount of connective tissue in center. The majority of acini contain colloid, others do not. Between acini there is fibrillar connective tissue with much lymphocytic infiltration; a number of acini have been destroyed by lymphocytes or connective tissue. The lymphocytic infiltration is in places moderate and in others more marked, though not overwhelming. Lymphocytes invade some acini. Grade 3.75. Here in family 13 is a very decided syngenesio-reaction. The piece was in a strange host during a period of 54 days. The marked reaction found in this case is in accordance with the fact that the second host, 16174 (13-18-9) had only 7 generations of brother-sister ancestry in common with either of the donors.

2. First transplantation. Cartilage transplanted from guinea-pig 13-21-19 (20898) to 13-20-17 (20761) 6 days.

Second transplantation, 13-20-17 to 13-23-1 (20986) 7 days. Duration of experiment 13 days altogether. The original cartilage

though showing areas of necrosis, also shows some newly-formed perichondrial cartilage. The fat tissue which is preserved is invaded in places by connective tissue. There is much fibrillar connective tissue around the cartilage. In the absence of a lymphocytic infiltration it is doubtful whether the connective tissue increase is to be considered as a specific reaction against the transplant.

3. First transplantation. Four thyroids from two guinea-pigs 32-19-8 (15634 and 15633) to guinea-pig 32-19-9 (16900) 37 days. The specimen is like an autotransplant or nearly so, with perhaps a slight increase in connective tissue.

Second transplantation, 32-19-9 (16900) to brother 32-19-9 (16899) 4 months, 9 days. Thyroid is like an autotransplant with low to medium-sized epithelium of acini which are close together and contain solid, much retracted colloid. There is a small amount of fibrous tissue in the center. The transplant is surrounded by fibrous tissue. The parathyroid is negative, although in the capsule around the parathyroid there are some lymphocytes. Grade 6 or 5.75. In this case the second transplantation was made into the brother of the first host. The total duration of experiment 2 was five and one-half months, and yet no definite reaction against the tissue has occurred. This demonstrates the great similarity of individuality differentials in this case. These experiments further demonstrate the feasibility of serial transplantations with as sensitive an organ as the thyroid gland, provided the individuality differentials of the hosts are similar to those of the donors.

SERIES E. *Multiple simultaneous transplantations in the same family, with examination within 6 weeks after transplantation.* In this and in the following series the variety of organs which were transplanted was enlarged; in addition to thyroid, parathyroid, and cartilage, such organs as spleen, adrenal, liver, pancreas, and bone, were also transplanted. In the first experiments four thyroids from two different donors were transplanted. It was of interest to test how more sensitive pieces of organs or tissues would behave under the favorable conditions in which the individuality differentials in host and donor are so similar to each other. Five experiments were made in this series.

1. 32-19-8 (15634) and 32-19-8 (15633) four thyroid lobes to 32-19-9 (16900)

*37 days.* Two thyroids examined. Both lobes behave like or almost like autotransplants, but there are collections of lymphocytes in lymph vessels and in various places in the fibrous tissue around the thyroid transplant. Grade 5.60.

2. Liver, spleen and pancreas from 13-19-9 (15648) to 13-18-10 (16787) *36 days.* The spleen shows lymph follicles in its periphery, pulp with blood sinuses and strands of hyaline connective tissue probably corresponding to trabeculae. Perhaps in the periphery of the capsule there is some lymphocytic infiltration. The lymph follicles have larger cells in their centers and smaller cells in the periphery. In the liver, the bile ducts are preserved and are actively proliferating, showing mitoses. They are surrounded by a fibrous capsule with slight lymphocytic infiltration. The liver tissue is well preserved, and apparent transitions between bile ducts and liver tissue are visible. Mitoses seem to occur in the liver cells. There are small collections of lymphocytes here and there in the liver tissue. The pancreas shows only fat tissue with an epitheloid, giant cell and lymphocytic infiltration. Grade about 5.50.

3. Adrenals from Y-4  $\left\{ \begin{array}{l} 13-20-2 \\ 13-19-15 \end{array} \right\}$  (16038) to 13-18-10 (16788) *36 days.* Normal adrenal tissue. There are cells containing yellow pigment; and fat cells are also present. In places, cholesterol crystals are surrounded by foreign body giant cells. There is fibrillar connective tissue in the periphery. No lymphocytic infiltration. Grade 6.

4. Two thyroids from 13-18-10 (16787) and one spleen from 13-21-11 (21161) to 13-21-19 (20897) *27 days.* Thyroids and parathyroids closely resemble autotransplants, showing very little lymphocytic infiltration in the center. Spleen contains Malpighian bodies with large endothelial cells in their centers. There are mitoses in and around lymph follicles; pulp with blood vessels and some connective tissue; mitoses in the endothelium of the sinuses; hemorrhage, and in the periphery of the transplant, collection of lymphocytes. Grade 5.25.

These experiments prove again that in family 13 a complete homogeneity of individuality differentials has not yet been attained. As in our previous experiments the individuality differentials resemble each other more on the average than the individuality differentials of brothers in non-inbred families. We find that spleen, adrenal, and liver tissue can be successfully transplanted in such



inbred families. Especially in the liver transplant, mitoses are found in bile ducts as well as in liver cells as late as 36 days after transplantation.

*SERIES F. Multiple simultaneous transplantations in the same family, examination taking place more than four months after transplantation.*

1. Thyroid, cartilage, bone, liver and adrenal from 32-20-9 (23396) to 32-20-7 (23588) 129 days. Thyroid, parathyroid, cartilage, bone marrow with megalokaryocytes preserved; bone and proliferating zone of cartilage near bone well preserved. Liver not definitely found. Grade 6.

2. Thyroid, cartilage, liver and adrenal from 13-23-3 (23525) to 13-21-13 (23448) 132 days. Thyroid, parathyroid, cartilage, bone, with zone of proliferating cartilage cells, bone marrow, with myelocytes, leucocytes, megalokaryocytes, well preserved; perhaps some slight connective tissue increase in bone marrow. No adrenal or liver found. Grade 6.

3. Thyroid, cartilage, adrenal, and liver from guinea-pig 13-23-5 (23525) to 13-21-3 (23660) 132 days. Thyroid: in center a considerable mass of lymphocytes. Some lymphocytes penetrate between acini. Also in fat tissue, a mass of lymphocytes. Connective tissue in center and elsewhere increased. Good ring of acini with good colloid. Cartilage resembles autotransplant; bone marrow well preserved. No definite liver or adrenal tissue found. Grade 4.75?

4. Thyroid, cartilage and adrenal from 32-20-9 (23396) to 32-19-6 (23601) 129 days. Thyroid: lymphocytic infiltration in center and fat tissue, which extends also between acini: parathyroid markedly infiltrated and destroyed by lymphocytes. Around cartilage perhaps very slight increase in fibrous tissue and a few lymphocytes. Proliferation zone and cartilage cells near bone preserved. Bone marrow preserved, (megalokaryocytes, leucocytes) but much lymphocytic infiltration and connective tissue increase in peripheral parts. No adrenal found. Grade 5.

5. Thyroid, adrenal and liver from 2-17-30 (23458) to 2-17-18 (23551) 132 days. Thyroid: acini close together with colloid; in addition numerous other acini without colloid and some of these compressed. Over large areas very small acini with colloid staining red with eosin. The acinus cells of the small acini may be destroyed. In many places the thyroid appears still like an autotransplant; but

an intense lymphocytic infiltration is beginning in other places, the appearance is that of a lymph gland and the thyroid is being destroyed. The lymphocytes infiltrate also the interstices between acini and later enter the acini. Apparently some remnants of liver in the form of yellow tissue; much fibrous tissue formation and dense masses of lymphocytes. Lymph vessels filled with lymphocytes. Grade 4. This case illustrates the secondary late infiltration and destruction of syngenesiotransplants by lymphocytes.

6. Thyroid, cartilage, adrenal and liver from 2-17-30 (23458) to 2-19-13 (23347) 132 days. No thyroid or liver found. Cartilage well preserved, but some lymphocytic collections around cartilage and in fat tissue; they are larger than they would be in autotransplants. Well preserved muscle tissue, probably transplanted. Tissue consisting of yellow vacuolar cells with nuclei, surrounded by fibrous tissue with lymphocytic masses. Tissue largely infiltrated and destroyed by lymphocytes. (Adrenal?) Grade 4.75?

7. Cartilage, ovary, liver, adrenal from 32-23-2 (23420) to 32-18-7 (21067) 129 days. Well preserved cartilage and bone, surrounded by fat tissue. No increase in connective tissue, no lymphocytes. Proliferating zone of cartilage cells near bone. Bone marrow preserved. Cartilage partly vacuolar and dissolved; no lymphocytes. Ovary; preserved follicles of all sizes and normal ova, follicles in stage of granular degeneration, some atretic follicles. Germ layer: fibrillar connective tissue and muscle tissue well preserved. Medullary ducts; almost normal ovary. Liver and adrenal not found. Grade 6.

8. Cartilage, ovary, spleen from 2N-31 (19998) to 2-17-23 (23428) 132 days. Cartilage well preserved surrounded by fibrillar connective tissue. Distinct lymphocytic infiltration around cartilage. Over wide areas no lymphocytic infiltration. Two ovaries with much fibrous tissue and dense lymphocytic infiltration; also fimbria epithelium with lymphocytic infiltration. Atretic follicles with zonae pellucidae. One of the two ovaries has no good follicles, the other has primordial follicles with normal ova, small follicles partly without ova, and medullary ducts, the epithelium of which has been penetrated by lymphocytes. Spleen consists of fibrous tissue with hemorrhage. Connective tissue grows into transplant. Apparently some reticulo-endothelial tissue left. There is lymphocytic infiltration. It is difficult to determine how much of these lymphocytic

masses are Malpighian bodies of the transplanted spleen and how much represents lymphocytic infiltration on the part of the host. Grade 4? It is interesting to compare experiments 7 and 8. In experiment 7, the transplants behaved like autotransplants and the ovaries are in excellent condition. In experiment 8, there is decided syngenesio-reaction with lymphocytic infiltration, affecting also the ovaries; here the condition of the ovaries is much inferior.

9. Cartilage, spleen, testicle, pancreas from 13-22-11 (23543) to 13-21-13 (20808) 132 days. Cartilage normal, surrounded by fat tissue; in places small collections of lymphocytes around vessels. Very small collections of lymphocytes next to perichondrium. Testicle; tubules surrounded by much lymphocytic infiltration; no spermatozoa. Sertoli cells preserved. Spleen; sinuses separated by connective tissue; lymph follicles with large endothelial cells, some showing mitoses. Hemorrhagic areas into which connective tissue grows. Several serous cysts with much lymphocytic infiltration. Grade 5.

In three of these nine experiments the grade was 6; namely in families 32 in two cases, in family 13 in one case. In six experiments the results corresponded to syngenesiotransplantation. Grade 5, in family 32 and 13. Grade 4.75 in family 2 and 13. Grade 4 in two transplantations in family 2.

In families 32, 2 and 13 there was therefore non-identity of individuality differentials. The correspondence in the behavior of different organs in the same experiment is of interest; our previous results are thus confirmed. Of interest also is the late invasion of these transplants by lymphocytes. The invasion can become overwhelming and in the end destroy the transplant. Thyroid, cartilage, bone, bone marrow, parathyroid and ovary can relatively easily be transplanted; somewhat less readily spleen. Liver and adrenals were usually not found four months or later following transplantation. While thus individuality differentials are not yet identical in these families, they have reached a stage where they have become very similar to each other within the same family.

## II. HOMOIOTRANSPLANTATIONS AS CONTROL EXPERIMENTS

Three kinds of control experiments were carried out.

A. *Transplantations from one inbred family to another inbred family.*

B. *Transplantations in control stock B.* These are guinea-pigs which originally are from the same stock from which the inbred families were derived, but in this B stock, matings as close as those between second cousins have been avoided.

C. *Transplantations from B stock to a totally unrelated stock obtained in St. Louis.* It will be possible to report on those control experiments very briefly because they behave like typical homoio-transplantations, on which one of us has reported previously on several occasions.

### SERIES A. *Exchange of tissue between two inbred families.*

1. From family 32 to family 2. *20 days.* Thyroid in part preserved, in part infiltrated and destroyed by connective tissue and lymphocytes. In dense fibrous tissue much lymphocytic infiltration. Also parathyroid infiltrated by lymphocytes. Cartilage: part of fat tissue replaced by fibrous tissue; lymphocytic infiltration in fat tissue and around cartilage. Grade 3.25.

2. From family 32 to family 13. *20 days.* Thyroid: only fibrous tissue. Cartilage: partly necrotic; surrounded by fibrous tissue. Lymphocytes around cartilage. Bone marrow replaced by fibrillar connective tissue. Grade 1.

3. From family 32 to family 2. *20 days.* Thyroid: intense lymphocytic infiltration, some small areas of thyroid preserved. Cartilage with some perichondrial regeneration. Intense lymphocytic infiltration and connective tissue around cartilage; only slight amounts of fat tissue preserved. Grade 2.5.

4. From family 32 to family 35. *21 days.* Thyroid: some compressed acini without colloid, a few acini with colloid. Fibrous tissue and much lymphocytic infiltration between acini. Fibrous tissue around cartilage; fat tissue with thickened septa. Distinct lymphocytic infiltration. Grade 2.75.

5. From family 32 to family 13. *21 days.* Thyroid not found. Cartilage with lymphocytic infiltration. Bone marrow transformed into myxoid connective tissue. Grade 1.5.

6. From hybrid 35 plus 32 to hybrid 39 plus 13. 35 days. No thyroid left. Cartilage surrounded by dense fibrous tissue; marked lymphocytic infiltration. Proliferative zone of cartilage near bone necrotic. Bone marrow replaced by fibrillar connective tissue and lymphocytes; capillaries with connective tissue penetrating into bone. Grade 1.

Average grade in this series is 2.

**SERIES B. *Transplantation in control stock B.***

1. B-16854 to B 16870. 24 days. No thyroid (fibrous tissue only); cartilage mostly surrounded by fibrillar connective tissue, with much lymphocytic infiltration. Grade 1.

2. B-16870 to B-16854. 24 days. Grade 1.

3. B-16854 to B-16870. 24 days. Grade 1.

4. B-16870 to B-16854. 24 days. Grade 1.

5. B-16853 to B-16872. 25 days. Thyroid: around necrotic connective tissue some acini surrounded by fibrous tissue with no, or very little, colloid. Lymphocytes infiltrate acini. Cartilage surrounded by fibrillar connective tissue and areolar tissue with fibrous septa. Lymphocytic mantle around cartilage. Grade 2.

6. B-15681 to B-15536. 25 days. Thyroid very small. In fibrous tissue, infiltrated with lymphocytes, are small lumina of acini with remnants of colloid. Epithelium hardly recognizable. Fibrillar connective tissue with lymphocytes surround these acini, which are in process of destruction; many have already been destroyed. Hyaline fibrous tissue in center. Cartilage preserved, surrounded by fibrous tissue with variable amounts of lymphocytes. Remnants of areolar tissue. Lymphocytes penetrate a little into peripheral cartilage. Grade 2.25.

Average grade, 1.37.

**SERIES C. *Transplantation from B stock to unrelated St. Louis stock.***

1. B to Bu. 40 days. Compressed remnants of thyroid; lymphocytic infiltration. Fibrous tissue around cartilage. Lymphocytes penetrate into periphery of cartilage. Grade 2.

2. B to Po. 20 days. Lymphocytes penetrate into periphery of perichondrium. Grade 1.

3. B to Po. 30 days. No thyroid. Lymphocytes penetrate in places into cartilage. Grade 1.25.

4. B to Bu. 40 days. Grade 1.

Average grade 1.31.

*Comment.* The differences between these three series of homoio-transplantation and the six series in which transplantations were carried out in the same family are very striking; in the former the total average grade is 1.6; in the latter series the average grade is 5.28. Of course, these figures can only claim to be approximate. We may state that the average grade in the transplantations within the same family is between 5 and 6, but nearer 5 than 6. There is an indication that families 32 and 13 are more strange to each other than families 32 and 2 or 32 and 35. However, this point needs further investigation. Of interest is also the marked reaction against tissues of hybrids in homoio-transplantation.

### III. TRANSPLANTATION FROM BROTHER TO BROTHER WITHIN AN INBRED FAMILY

When, in an ordinary non-inbred strain, transplantations from brother to brother are carried out, the results are better than in ordinary homoio-transplantation, or in syngenesiotransplantation between children and parents or *vice versa*.<sup>1</sup> It was of interest to determine whether in case of brother to brother transplantations within the inbred family the results would equal those of autotransplantation, and whether the individuality differentials between brothers in the inbred family had become identical.

We carried out two series of experiments in this direction; in the first one, series A, we used individuals belonging to the same family and in series B we exchanged tissues between brothers which were hybrids, the parents belonging to different inbred families.

#### SERIES A:

1. From 35-23-8 (16715) to 35-23-8 (16716) 5 months, 16 days. Thyroid and parathyroid behave like autotransplants. Grade 6.
2. From 32-19-9 (16900) to 32-19-9 (16899) 4 months, 9 days. Grade 6 (included among successive transplantations).
3. From 2N-26 (15673) to 2N-26 (15674) 61 days. Grade 6. Thyroid with auto-structure, acini close together. Solid retracted colloid, medium-sized epithelium. In one place there is a small number of lymphocytes. Cartilage well preserved, surrounded by areolar and fat tissue. No lymphocytes.

4. From Y-4  $\left\{ \begin{array}{l} 13-20-2 \\ 13-19-5 \end{array} \right\}$  (16039) to Y-4  $\left\{ \begin{array}{l} 13-21-2 \\ 13-19-5 \end{array} \right\}$  (16038) 40 days.

Transplant with striated muscle well preserved. Grade 6. (The corresponding transplantation to a relative a little further removed produced a syngenesio-reaction.)

5. Thyroid; cartilage and salivary gland from 2N-20 (15618) to 2N-20 (15619) 39 days. Grade 6.

6. From 2-18-4 (15693) to 2-18-4 (15692) 49 days. No connective tissue, new formation, nor lymphocytes. Grade 6.

7. From 13-19-9 (15649) to 13-19-9 (15648) 35 days. Thyroid with auto-structure, but in places in center and between acini around vessels are masses of lymphocytes surrounding and destroying acini. In a corresponding transplant in more distantly related animals of the same kind, there is much more lymphocytic infiltration than in brother to brother transplantation. Cartilage partly necrotic. Regenerating perichondrial cartilage infiltrates and replaces the necrotic cartilage. Connective tissue grows as papillae into necrotic cartilage, and the individual connective tissue cells grow also singly into it. Bone seems to have been produced in places where there was necrotic cartilage. In areolar tissue around cartilage some increase in connective tissue. Some megalokaryocytes, new bone marrow. Grade 5.

8. From 32-17-10 (15611) to 32-17-10 (15609) 40 days. Grade 6.

9. From 32-17-11 (15575) to 32-17-11 (15574) 10 days (died). Grade 6.

10. From 2-17-10 (15959) to 2-17-10 (15960) 50 days. Grade 6.

11. From 2-17-5 (15695) to 2-17-5 (15696) 30 days. Grade 6.

12. From 2-17-5 (15695) to 2-17-5 (15694) 30 days. Grade 6.

13. From 2N-20 (15617) to 2N-20 (15616) 12 days (died). Thyroid: very little lymphocytic reaction, but in places around the vessels there are small collections of lymphocytes. Cartilage similar to autotransplant. Grade 5.25.

14. From 2N-20 (15617) to 2N-20 (15619) 45 days. Grade 6.

*Comment.* In these 14 experiments the examinations were made after *five months, sixteen days; four months, nine days; 61, 50, 49, 45, 40, 40, 39, 35, 30, 30, 12 and 10 days.* In twelve cases the grade was 6, which means that the pieces showed the character of autotransplants; the individuality differentials between brothers in the same inbred family were identical as far as this test indicates. But this

statement is correct only with the reservation that possibly a reaction might still have occurred, if the pieces had been allowed to stay in the host for a still longer period of time. Therefore, we can only say definitely that in these twelve cases the individuality differentials of brothers closely approached or perhaps reached the condition found in autotransplant. The families in these twelve cases were: 35, one case; 32, three cases; 2, six cases; 13, two cases. In one case in family 13 there was a distinct syngenesio-reaction (Grade 5) and in one case in family 2 there was probably a very mild syngenesio-reaction (Grade 5.25). It is again family 13 in which individuality differentials are so distinctly divergent that even against the tissues of brothers a reaction is elicited; probably also in family 2N identity between the individuality differentials of brothers has not yet been entirely reached. The latter result is in accordance with the mixed breeding (within family 2) of mating 2N-20. The average grade in this series was 5.87 or 5.92 if experiment 13 is omitted.

SERIES B. 1. C-I-58  $\left\{ \begin{array}{l} 13-13-16 \\ 32-14-13 \end{array} \right\}$  (16050) to  
 C-I-58  $\left\{ \begin{array}{l} 13-13-16 \\ 32-14-13 \end{array} \right\}$  (16052)

40 days. Two transplanted lobes of thyroid with structure of autotransplants. Well preserved parathyroid. Marked lymphocytic infiltration in center of thyroid and in parathyroid, and some infiltration between acini. Lymphocytes have infiltrated and destroyed a great part of areolar tissue. Cartilage and striated muscle tissue well preserved, surrounded by good areolar tissue; connective tissue penetrates into necrotic cartilage; around vessels and cartilage in places a slight mantle of lymphocytes. Grade 5. We have here to deal with the F<sub>2</sub> generation of hybrids between families 13 and 32; this fact may explain the lack of auto-reaction in this case.

2. C-O-237  $\left\{ \begin{array}{l} 39-14-10 \\ 2-13-7 \end{array} \right\}$  (15891) to C-O-237  $\left\{ \begin{array}{l} 39-14-10 \\ 2-13-7 \end{array} \right\}$  (15893)

35 days. Thyroid and cartilage about like autotransplants. Very slight lymphocytic collections in center which are probably still within the range of that found in autotransplants. Grade about 6.

3. C-O-237  $\left\{ \begin{array}{l} 39-14-10 \\ 2-13-7 \end{array} \right\}$  (15891) to C-O-237  $\left\{ \begin{array}{l} 39-14-10 \\ 2-13-7 \end{array} \right\}$  (15894)

35 days. No lymphocytes. No increase in connective tissue. Grade 6.



4. C-O-268  $\left\{ \begin{matrix} 32-16-9 \\ 39-14-17 \end{matrix} \right\}$  (16023) to C-O-268  $\left\{ \begin{matrix} 32-16-9 \\ 39-14-17 \end{matrix} \right\}$  (16025)

35 days. Thyroid and cartilage. Grade 6.

5. C-O-252  $\left\{ \begin{matrix} 13-14-13 \\ 32-15-14 \end{matrix} \right\}$  (16795) to C-O-252  $\left\{ \begin{matrix} 13-14-13 \\ 32-15-14 \end{matrix} \right\}$  (16794)

25 days. Thyroid approaching autotransplant; some slight diffuse lymphocytic infiltration in center; also in small peripheral area some lymphocytes. In places around cartilage a slight amount of newly formed connective tissue. Grade 5.5.

6. Reciprocal to (5): 16794 to 16795. Thyroid and cartilage, 25 days. Grade 6.

7. C-O-234  $\left\{ \begin{matrix} 2-13-7 \\ 35-16-20 \end{matrix} \right\}$  (17078) to C-O-234  $\left\{ \begin{matrix} 2-13-7 \\ 35-16-20 \end{matrix} \right\}$  (17079)

35 days. Thyroid like autotransplant. A small number of lymphocytes around vessels in center, probably within the range of that found in autotransplantation. Cartilage like autotransplant. Grade 6.

8. C-O-285  $\left\{ \begin{matrix} 35-21-2 \\ 32-15-15 \end{matrix} \right\}$  (16756) to C-O-285  $\left\{ \begin{matrix} 35-21-2 \\ 35-15-15 \end{matrix} \right\}$  (16757)

26 days. Thyroid like autotransplant; cartilage perhaps with slightly increased connective tissue and with some lymphocytes. Grade 5.75?

*Comment.* In these eight experiments the time of examination varied between 40 and 25 days. In five experiments the grade was 6. The hybrids in these cases were combinations of 39 plus 2; 39 plus 32; 13 plus 32; 2 plus 35. In three cases the transplants were not identical with autotransplants. The combinations and grades in these cases were as follows: 13 plus 32, grade 5; 13 plus 32, grade 5.5; 35 plus 32, grade 5.75. In the case of the last of these hybrids, experiment 8, it is doubtful whether grade 6 has not been reached. In experiments 5 and 6 (hybrid 13 plus 32) reciprocal transplants did not give exactly the same result, although the reactions in both cases were similar. It is again those hybrids, into whose composition family 13 entered, that do not yet reach the identity of individuality or 5.91 if we exclude experiments III A-13 and III B-1 differentials in brothers. The average grade in this series is 5.78, 5.89 if we omit experiment III B-1, or 5.91 if we exclude experiments III A-13 and III B-1. The average grade in series A and B combined is 5.84. These average grades also indicate that a perfect identity of the individuality differentials has not yet been reached

in all cases, although on the whole they closely resemble one another. The figure for the average is here higher than in the case of the exchange of tissues in the same family between individuals which belonged to the same inbred family, but were not nearly related. In the latter case the average grade was 5.28.

#### IV. SYNGENESIOTRANSPLANTATIONS (BROTHER TO BROTHER) IN NON-INBRED FAMILIES

We have previously analyzed syngenesiotransplantation in non-inbred families of guinea-pigs. We found as the average grade of brother to brother transplantation 3.6. We shall here briefly state the result of brother to brother transplantation in the B strain of guinea-pigs for comparison with the results obtained in brother to brother transplantation within inbred families.

There were fifteen experiments; examination took place 25 to 40 days following transplantation. Grades: 6; 5.5; 5; 5; 5; 4.75; 3.75; 3.25; 3; 2; 2; 2; 1; 1; 1. Average grade 3.35.

A few examples may suffice. 1 B-202 to B-202, 35 days. No thyroid found. Fibrous tissue surrounds cartilage which is partly cellular. Marked lymphocytic mantle around cartilage, but in places loose connective tissue with fewer lymphocytes. The latter penetrate into cellular cartilage. Epithelioid reaction in fat tissue.

2. B-274 to B-274. 40 days. Thyroid very well preserved; good acini, close together with good solid restricted colloid. A little connective tissue in center; also a little areolar tissue with a small number of lymphocytes in one place in center. Cartilage well preserved surrounded by areolar tissue; no lymphocytes. Grade 6.

3. B-261 to B-261. 35 days. Thyroid. Remnants of acini, some containing colloid, surrounded by a mass of lymphocytes. Apparently remnants of parathyroid infiltrated by lymphocytes; especially in center of parathyroid much lymphocytic infiltration. Periphery of ring of acini surrounded by lymphocytes. Well preserved cartilage; where it is thicker, it is partly shrunken. Cellular cartilage is surrounded by areolar tissue which includes strands of connective tissue and lymphocytes, especially near the perichondrium and around vessels. However, the greater part of areolar tissue contains no lymphocytes. Grade 3.25.

*Comment.* The average grade for the syngenesiotransplants (brother to brother transplantations in non-inbred families) is con-

siderably lower than the average grade of ordinary homoio-transplantations in the inbred families and still lower than the grade for the brother to brother transplantations in the latter.

#### V. TRANSPLANTATION FROM HYBRID TO PURE COMPONENT FAMILY

In this series a male belonging to one of the inbred families and a female belonging to a different family were mated, and the hybrid thus obtained was used for transplantation to another individual who was not related to the hybrid but belonged either to the family of his father or mother. Thus twenty-four experiments were carried out. The length of time during which the pieces were left in the host varied between 37 and 20 days. In one additional case the host died 15 days after transplantation.

The relationships between host and donor will be brought out more distinctly in the following list of experiments:

1. C-O-282  $\left\{ \begin{array}{l} 2-15-3 \\ 32-17-7 \end{array} \right\}$  (15566) to (32-17-11 (15575) 24 days.

Grade 4.25. Thyroid: structure of autotransplant, but dense lymphocytic masses in center and in places in peripheral fibrous capsule around vessels. Lymphocytes penetrate from center towards periphery between acini. Many acini have been destroyed. Some connective tissue increase in center and around acini. Cartilage: well preserved surrounded by areolar tissue with slight increase in connective tissue and lymphocytes.

2. Same to 2N-20 (15618) 24 days. Grade 4.

3. C-O-298  $\left\{ \begin{array}{l} 13-21-4 \\ 2-16-17 \end{array} \right\}$  (18188a) to 13-20-13 (15784) 35 days. Grade 2.

4. Same to 2-16-17 (18059) 35 days. Grade 1.

Thyroid: only fibrous tissue found. Cartilage: with a great deal of necrosis; some shrunken cells. Much connective tissue and moderate mantle of lymphocytes around cartilage, the necrotic parts of which are entered by some connective tissue and lymphocytes.

5. C-O-304  $\left\{ \begin{array}{l} 32-19-10 \\ 2-15-16 \end{array} \right\}$  (18305) to 32-18-15 (16753) 37 days. Grade 3.75.

6. Same to 2N-24 (18043) 37 days. Grade 3.25.

Thyroid: very good acini, close together in places and with solid retracted colloid. Much lymphocytic infiltration around and between acini. Also in center of thyroid lymphocytes surround some

acini and penetrate into thyroid in places; lymph vessels filled with lymphocytes. The greater part of the thyroid is intact, but in the connective tissue around acini there is much lymphocytic infiltration. Lymphocytes penetrate also into colloid. There are areas of fibrous tissue, of areolar tissue and of lymphocytic infiltration around cartilage. Striated muscle tissue preserved at one end.

7. C-O-240  $\left\{ \begin{array}{l} 32-16-9 \\ 35-16-11 \end{array} \right\}$  (15943) to 32-19-9 (16901) 37 days. Grade 4.
8. Same to 35-23-8 (16716) 37 days. Grade 2.
9. C-O-297  $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$  (18063) to 35-12 (18589) 25 days. Grade 2.50.
10. Same to 2N-25 (18184) 25 days. Grade 3.
11. C-O-282  $\left\{ \begin{array}{l} 2-15-3 \\ 32-17-7 \end{array} \right\}$  (15567) to 32-19-8 (15633) 25 days. Grade 6.
12. Same to 2-17-5 (15696) 25 days. Grade 3.
13. C-O-237  $\left\{ \begin{array}{l} 39-14-10 \\ 2-13-7 \end{array} \right\}$  (15893) to 39-16-21 (15727) 25 days. Grade 2.5.
14. Same to 2-18-4 (15692) 25 days. Grade 2.85.
15. C-O-297  $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$  (18066) to 2N-31 (19998) 20 days. Grade 2.75.

Much thyroid tissue destroyed, relatively little thyroid left. The remaining ring of acini with much lymphocytic infiltration and much fibrous tissue formation. Around cartilage still some muscle tissue preserved.

16. Same to 35-22-17 (17966) died after 15 days. Grade 3.25. (Some muscle tissue preserved.)

17. C-O-298  $\left\{ \begin{array}{l} 13-21-4 \\ 2-16-17 \end{array} \right\}$  (18190) to 13-21-8 (18185) 21 days.

Grade 1.25. Thyroid destroyed; merely dense fibrous tissue and some lymphocytes surrounded by fat tissue and epithelioid and giant cells. Bone marrow replaced by loose connective tissue. Cartilage mostly necrotic. Some fat tissue mingled with epithelioid and giant cells. Over wide areas cartilage surrounded by connective tissue with some lymphocytic infiltration. Near bone, a zone of cartilage is living.

18. Same to 2N-32 (20338) 21 days. Grade 2.

19. C-O-269  $\left\{ \begin{array}{l} 32-16-9 \\ 39-14-17 \end{array} \right\}$  (16025) to 32-19-8 (15633) 23 days.

Grade 5.5. Large thyroid with solid retracted colloid; medium-sized

epithelium. Many large acini. A small amount of connective tissue in center. No lymphocytes except in one connective tissue septum, where there is a slight collection. Well preserved parathyroid. Cartilage well preserved, surrounded by areolar tissue. There is a slight increase in connective tissue with very little lymphocytic infiltration in areolar and fat tissue.

20. Same to 39-16-21 (15633) 23 days. Grade 1.25.  
 21. C-O-304  $\left\{ \begin{array}{l} 32-19-10 \\ 2-15-16 \end{array} \right\}$  (18306) to 2N-25 (18182) 29 days. Grade 5.5.  
 22. C-O-284  $\left\{ \begin{array}{l} 39-13-13 \\ 32-17-5 \end{array} \right\}$  ( ) to 39-16-21 (15727) 25 days. Grade 3.25.  
 23. Same to 32-17-11 (15574) 25 days. Grade 5.  
 24. C-O-297  $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$  (17093) to 2-17-5 (15696) 31 days. Grade 4.

The hybrid combinations used were as follows:  $\frac{2}{32}$  to 2 in four different experiments;  $\frac{2}{32}$  to 32 in three different experiments.

(1)  $\frac{2}{32}$  to 32, 24 days. Grade 4.25. (2)  $\frac{2}{32}$  to 2N, 24 days.

Grade 4. (5)  $\frac{32}{2}$  to 32, 37 days. Grade 3.75. (6)  $\frac{32}{2}$  to 2N, 37 days.

Grade 3.25. (11)  $\frac{2}{32}$  to 32, 25 days. Grade 6. (18)  $\frac{2}{32}$  to 2. 25 days.

Grade 3. (21)  $\frac{32}{2}$  to 2N, 29 days. Grade about 5.5.

$\frac{13}{2}$  was used in four experiments. (3)  $\frac{13}{2}$  to 13, 35 days. Grade 2.

(4)  $\frac{13}{2}$  to 2, 35 days. Grade 1. (16)  $\frac{13}{2}$  to 13, 21 days. Grade 1.5.

(17)  $\frac{13}{2}$  to 2N, 21 days. Grade 2. In transplantations in which family 13 enters as a component of a hybrid, the reactions against the transplants are more severe than in transplantations in which family 32 takes the place of family 13.

$\frac{32}{35}$  was used in two experiments. (7)  $\frac{32}{35}$  to 32, 37 days. Grade 4.

(8)  $\frac{32}{35}$  to 35, 37 days. Grade 2.

$\frac{2}{35}$  was used in five experiments. (9)  $\frac{2}{35}$  to 35, 25 days. Grade 2.50. (10)  $\frac{2}{35}$  to 2N, Grade 2. (14)  $\frac{2}{35}$  to 2N, 20 days. Grade 2.75. (15)  $\frac{2}{35}$  to 35 (died after 15 days). Grade 3.25. (24)  $\frac{2}{35}$  to 2, 31 days. Grade 4. In these experiments the results were almost but not quite as unfavorable as in transplantation for hybrid  $\frac{13}{2}$ .

$\frac{39}{2}$  was used in two experiments. (12)  $\frac{39}{2}$  to 39, 25 days. Grade 2.5? (13)  $\frac{39}{2}$  to 2, 25 days. Grade 2.75-3. In this combination the results were likewise unfavorable.

Hybrid  $\frac{32}{39}$  was used in four experiments. (19)  $\frac{32}{39}$  to 32, 23 days. Grade 5.5. (20)  $\frac{32}{39}$  to 39, 23 days. Grade 1.25. (22)  $\frac{39}{32}$  to 39, 25 days. Grade 3.25.  $\frac{39}{32}$  to 32, 25 days. Grade 5. In this combination the results were relatively favorable.

In three experiments, the result approaches that in autotransplantation; in one experiment the result corresponded to a favorable syngenesiotransplantation. In nine cases the result was a very pronounced syngenesio-reaction. In three cases the result was on the border-line between a syngenesio- and homoio-reaction and in eight experiments a homoio-reaction was obtained. The average grade was 3.25. The average grade was therefore only very slightly less favorable than that obtained in brother to brother transplantation in non-inbred families.

We see thus that the grades in the different experiments vary as much as between 6 and 1. The grades were as follows: 6; 5.5; 5.5; 5; 4.25; 4; 4; 4; 3.75; 3.25; 3.25; 3.25; 3; 3; 2.85; 2.75; 2.50; 2.50; 2; 2; 2; 1.5; 1.25; 1.

In eleven experiments we transplanted the organs of one hybrid to both the component parent strains; in none was the transplantation made to the direct parents of the hybrids. In seven of these experiments the results were similar after transplantation into both parent strains, in three experiments they were very different, and in

one case the difference, although noticeable, was not quite so pronounced. Different results were obtained in the following combinations:  $\frac{2}{32}$  to 32, grade 6.  $\frac{2}{32}$  to 2 grade 3.  $\frac{32}{35}$  to 32, grade 4.  $\frac{32}{35}$  to 35, grade 2.  $\frac{32}{39}$  to 32, grade 5.5.  $\frac{32}{39}$  to 39, grade 1.25.  $\frac{39}{32}$  to 39, grade 3.25.  $\frac{39}{32}$  to 32, grade 5.

In general it seems that hybridizations, into which family 32 enters as one of the strains, are relatively favorable; the reactions on the whole are slight, at least in a number of these cases, and especially when the host belongs to family 32. The presence of a single dose of 2, 35 or 39 family-differential does not necessarily lead to severe reactions if a guinea-pig belonging to family 32 is the host, but in other cases it may do so. If a member of family 2 is host, the reactions against hybrid tissue  $\frac{32}{2}$  are usually more marked than the reactions on the part of a 32 host, but here also they may be very slight. Similarly host 32 reacts much less actively against  $\frac{32}{35}$  hybrid tissue than a 35 host. In host 35 the reactions are pronounced against hybrid tissue of 35 with 2 or 32. Host 32 showed relatively slight reactions against  $\frac{32}{39}$  hybrid tissue, while host 39 showed decidedly more marked reactions against the same tissue. Host 2 showed quite noticeable reactions against  $\frac{2}{35}$  and  $\frac{2}{39}$  hybrid tissues. Among the most marked reactions were those obtained against hybrid tissue in which 13 entered as a component. The reactions occurred in host 2 as well as in host 13. Throughout our experiments we found the most marked reaction within family 13, which represents probably the least homozygous of the various families. Not only is the reaction against family 13 most marked on the part of other families, but family 13 as host reacts likewise most actively against the individuality differential of the other families. We may assume that the different families show a varying degree of relationship to each other, and that while the strange genes in general cause a reaction on the part of the host, perhaps certain genes call forth a more severe reaction than others, and furthermore that certain

families are possibly less sensitive to differences in genes than other families. The experiments recorded so far in these series suggest these conclusions.

#### VI. TRANSPLANTATION FROM PURE COMPONENT FAMILY TO HYBRID

In this series the reverse transplantations were carried out. Tissues were transferred to a hybrid from an individual belonging to one of the two inbred families, members of which had been used for hybridization. Host and donor were not directly related to each other, except in so far as all members of an inbred family are in certain respects so related.

Fourteen experiments were carried out in this series; the period during which the transplants remained in the host varied in the different cases between 25 and 35 days.

The following experiments were made:

1. 2N-25 (18184) to C-O-297  $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$  (18063) 25 days.

Grade 6. Thyroid: with auto-structure; acinus cells of low to medium height, solid retracted colloid. Some loose connective tissue and large vessels in center. No lymphocytes, no increase in connective tissue. Cartilage well preserved; normal fine fibrillar connective tissue and fat tissue surround the cartilage. Again no lymphocytes; no increase in connective tissue.

2. 35I-2 (18589) to same hybrid, 27 days. Grade 5.25. Thyroid, as in preceding experiment, but some masses of lymphocytes penetrate from outside to center. Cartilage also well preserved and surrounded by fat tissue, but in places some fibrillar connective tissue with a few lymphocytes around it. In this case a very slight reaction took place.

3. 2-16-17 (18059) to C-O-298  $\left\{ \begin{array}{l} 13-21-4 \\ 2-16-17 \end{array} \right\}$  (18188a) 35 days. Grade 6.

4. 13-21-13 (15784) to same hybrid. Grade 6.

5. 32-18-15 (16753) to C-O-304  $\left\{ \begin{array}{l} 32-19-10 \\ 2-15-16 \end{array} \right\}$  (18305) 35 days.

Grade 6. Only a small collection of lymphocytes around foreign body; otherwise like autotransplant.

6. 2N-24 (18043) to same hybrid. 35 days. Grade 6. As in the majority of other transplants, the perichondrium produces in places



new cartilage, which either penetrates adjoining necrotic cartilage and replaces it or which is deposited in the form of a plate.

7. 32-19-9 (16900) to C-O-240  $\left\{ \begin{array}{l} 32-16-9 \\ 35-16-11 \end{array} \right\}$  (15943) 35 days.

Grade 6. Transplants resemble autotransplants. Into peripheral necrotic cartilage some connective tissue is growing and in places it penetrates superficially even adjoining living cartilage. A very few small strands of lymphocytes in fat tissue; conditions are probably still within the range of those observed in autotransplants.

8. 35-23-8 (16716) to same hybrid. 35 days. Grade 5.75. Thyroid and parathyroid like autotransplants, except that we find in the parathyroid in one place a slight, but distinct collection of lymphocytes which exceeds in size collections found in cases of autotransplantation. In areolar and fat tissue around cartilage there are few polymorphonuclear leucocytes and lymphocytes. There may also possibly be here and there a very slight increase in connective tissue.

9. 32-18-9 (18179) to C-O-304  $\left\{ \begin{array}{l} 32-19-10 \\ 2-15-16 \end{array} \right\}$  (18306) 25 days. Grade 6.

10. 2N-25 to same hybrid 25 days. Grade 6. Thyroid transplant consists of ring of well preserved acini with a small amount of connective tissue and areolar tissue in center. Colloid in acini is solid and somewhat retracted. Cartilage is surrounded by areolar and fat tissue.

11. 13-18-10 (16788) to C-O-298  $\left\{ \begin{array}{l} 13-21-4 \\ 2-16-17 \end{array} \right\}$  (18190) 32 days.

Grade 5.50. Thyroid, parathyroid and cartilage like autotransplants, except for a collection of lymphocytes in periphery of parathyroid which also penetrates into this gland. Cartilage is well preserved, but parts are necrotic; the latter are surrounded by regenerated perichondrial cartilage which as usual consists of small cartilage cells with nuclei that are more prominent than in older cells, its blue stain contrasting within the light stain of the cytoplasm. In other places the host connective tissue grows into necrotic cartilage and replaces it. Some lymphatics in fat tissue are filled with lymphocytes.

12. 2N-25 (18182) to same hybrid. 32 days. Grade 5.50. Thyroid like autotransplant except that in one place in the center there is a small mass of lymphocytes which probably exceeds in size the

range of lymphocytic collections found in autotransplants. In the cartilage, some regenerated transplanted muscle tissue with chains of nuclei. There is a very slight increase of connective tissue with some lymphocytes found in the areolar and fat tissue around cartilage.

13. 2N-20 (15619) to C-O-297  $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$  (18066) 31 days.

Grade 5.50. Thyroid like autotransplant, except for a few small collections of lymphocytes. In cartilage transplant there is some transplanted striated muscle with only a few nuclear chains. In places there are small collections of lymphocytes around vessels in fat tissue of cartilage transplant probably exceeding the number found in autotransplants.

14. 35-22-17 (17695) to same hybrid. 31 days. Grade 5.25. Thyroid resembles autotransplant except that there is some thickening of connective tissue septa and a slight lymphocytic infiltration. In cartilage transplant, which is well preserved, there are some distinct collections of lymphocytes in areolar and fat tissue and there is here also a very slight increase in connective tissue. Areas of necrotic cartilage are partly replaced by perichondrial cartilage which may regenerate in the form of a plate. Near the junction of xiphoid cartilage with bone, connective tissue grows into areas of necrotic cartilage and produces a bone-like substance. We find also fibrillar bone marrow with megalokaryocytes, capillaries and lymphocytes. Osteoclasts also are seen at the edge of bone marrow. Between the areas of bone, perichondrium forms cartilage. A somewhat similar condition was probably observed in experiment (8) of this series. In this transplant very well formed muscle tissue produced by transplanted muscle cells was seen.

*Comment.* The average grade in these fourteen experiments is 5.77, approaching therefore the condition found in autotransplantation. In eight cases the grade corresponds to the result obtained in autotransplantation. In the remaining experiments the grades are 5.75; 5.50; 5.50; 5.50; 5.25; 5.25. Grade 6 was obtained in the following transplantations: 2 to  $\frac{2}{35}$ ; 32 to  $\frac{32}{2}$  in two experiments; 2 to  $\frac{32}{2}$  in two experiments; 32 to  $\frac{32}{35}$ ; 13 to  $\frac{13}{2}$  and 2 to  $\frac{13}{2}$ . The other grades were obtained in the following combinations: 35 to  $\frac{2}{35}$  in two

experiments 2 to  $\frac{2}{35}$ ; 13 to  $\frac{13}{2}$  and 2 to  $\frac{13}{2}$ . Slight reactions were therefore observed in cases in which family 13 or family 35 were hosts or donors. The strongest reactions were obtained in cases in which 35 was both host and donor, and especially in experiments in which family 35, serving as host, was combined with family 2. When families 32 and 35 were combined as host and donor a reaction, although somewhat mitigated, was observed. As in the preceding series we found that hybridizations, in which families 13 and 35 were involved, gave rise to relatively stronger reactions if tissues were exchanged between hybrids and component families than if the transplantations concern families 32 and 2. In this series we have not used family 39. On the other hand, we find that exchange of tissues in cases in which families 13 and 35 enter may not necessarily give rise to reactions in all cases; the individuality differentials between donor and host may be so constituted that no reaction occurs, at least within the range of time used in the particular experiment.

These fourteen experiments may be arranged in seven groups in which an exchange of tissue took place between hybrid members of the two families in each case. In four of these groups the results were about the same without regard to the character of the donor family; in three there was some difference, in each instance the stronger reaction was obtained when family 35 furnished the donor.

If we compare the average grades in the different series, omitting those in which the number of experiments at present is still very small, we find in the transplantations within the same family an average of 5.6 or slightly higher. The multiple transplantations extending over a long period of time give a somewhat lower average, namely 5.4, as do successive transplantations, although the number of our experiments is very small in this latter subseries. Some complicating factors may enter in the case of multiple transplantations extending over a long period of time, and of successive transplantations.

In experiments in which we transplanted from one hybrid to another hybrid of the same composition, not directly related, stronger reactions seemed to be elicited, and the averages were correspondingly lower, namely grade 4; but here again the number of our experiments is as yet too small.

The average grade of 5.6 in the uncomplicated subdivision of this

series is slightly lower than the average we obtained in the last series in which we transplanted pieces from a component strain to a hybrid; here the grade was 5.77. Of course the slight difference between these figures may have no significance. The figure for the last series is about the same as that for the series in which tissues were exchanged between two brothers, which were both hybrids of the same inbred families; here the average grade is 5.78. In this case the presence of two component families in donor as well as in host is an unfavorable condition which is compensated by the fact that both host and donor are brothers. In general we have some indication that transplantations from one hybrid to another not directly related hybrid, both derived from the same families, call forth a stronger reaction and give a lower average grade (tentatively 4, a figure based however on a very small number of experiments) than transplantations within the same inbred family, if host and donor are not directly related. Transplantations from brother to brother in the inbred families are closer to autotransplantations than any of the preceding kinds of transplantations, the average grade being 5.9; this of course is in accordance with expectations. On the other hand, the transplantations from a hybrid to the component strains call forth strong reactions; the average grade is accordingly low, namely 3.25, which is very similar to the average grade in brother to brother transplantations in the B group, in which the average is 3.35.

#### CONCLUSIONS

From these results we may conclude that it is not the similarity or difference between individuality differentials of donor and host which determines the reaction against the transplant, but the reaction depends on the presence in the host of genetic factors of the donor. The lack in the donor of genetic factors present in the host is apparently of little or no consequence. In the case of tumor transplantations in different varieties of mice which have been inbred, although as it seems not exclusively through consecutive brother to brother matings, Little and Tyzzer<sup>3</sup> found that transplantations from hybrid to parent strain gave negative results as far as the number of takes was concerned, while the reverse transplantation gave 100 per cent takes. They concluded therefore that only one dose of genes is required for successful transplantation. We found in our experiments, in which we used a much finer means of measuring genetic

composition than tumor transplantations, that in different transplantations from hybrid to component strain as well as from component strain to hybrid, individual differences exist which are very pronounced in the case of transplantations from hybrid to component strain. It is probably not so much the presence of a single dose of the genes which is required, as far as the absence of reaction against the transplant is concerned, as the lack of a strange gene in the donor; while the presence of a strange gene in the host is without significance. Thus the transplantation from one hybrid to another hybrid is more injurious than transplantation from component family to hybrid.

More recently, Little and Johnson<sup>4</sup> found in an inbred strain of Japanese waltzing mice that transplantation of pieces of spleen from waltzing mice to hybrids of these animals with white mice, behaved like autotransplants; while spleen transplanted from such hybrids to waltzing mice behaved like very pronounced homoiotransplants, and were destroyed within a short time. In these experiments the transplantations in which the hybrids served as hosts gave much better results than the reciprocal transplantations; but again the lack of finer means of measuring genetic differences led in all probability to incorrect estimates of the genetic identity or lack of identity in the individuality differentials of host and donor. It is very probable that the results in these transplantations did not correspond exactly to those found in autotransplantation, or to those of complete homoiotransplantation, but that both were intermediate between these two extremes. However the fact brought out by Little and Johnson and also, it seems, their interpretation of these facts, appear to be in essential harmony with the conceptions which one of us had previously developed concerning the individuality differential.

The results which we obtained cannot be considered inevitable from *a priori* reasoning. Thus one might have expected that the presence in the individuality differential of the host of a set of genes which are not present in the donor and transplanted tissue should lead to essential differences between the metabolism of host and transplant and consequently to aggressive reactions on the part of the host; this evidently does not take place. It seems to be merely the presence of strange genes in the transplanted tissue which acts as a stimulus on the host and incites the cells of the latter to react.

Apparently the absence of genes in the transplant is without significance. It is the presence of strange genes in the transplanted tissue which causes the inferiority in the latter in respect to the survival and regenerative power.

Both of these consequences of transplantations of tissues, in which the individuality differentials are more or less incompatible with those of the host, agree with those established previously by one of us in his series of transplantations. The lymphocytic and connective tissue reactions, the behavior of blood vessels, the degeneration of sensitive tissues like bone marrow, liver cells and spleen tissue in accordance with incompatibilities of individuality differentials are the same in these experiments as those previously described.

As to other general conclusions, we may refer to the discussions given at the end of the various sections in this paper. We may again state that our observations point to the conclusion that in some families a homozygous condition has been reached more completely than in others, but that it has not yet been actually attained in any of these inbred families. It is probable that some families are more nearly related to each other than others and furthermore it is possible that certain strange factors present in some families and individuals cause a greater incompatibility reaction than others and, in addition, we must consider the possibility that the sensitivity and degree of reaction of certain families or individuals against individuality differentials surpass those of others.

There is some indication that in the transplantations within the same family, the individuality differentials of host and transplant are more similar to each other the larger the number of brother and sister matings which both individuals had in common before the separation of brother and sister matings in different sidelines and the smaller the number of these separate and distinct brother and sister matings for each of the two individuals in the preceding generations. Thus in family 2, three transplantations with an average grade of 4.6 were separated for twenty-four, twenty-four and twenty generations of brother and sister matings respectively; while preceding their separate matings they had six, six and nine common ancestral matings. In two other experiments in this family in which host and donor were separated for twelve and three generations only, and twelve and sixteen brother and sister matings in common, the grade was 6.

A similar result was obtained in matings in family 13. Here the average grade was 4.9 in a series of transplantations in which the number of generations of brother and sister matings since the splitting off of the sidelines of brother and sister matings varied between twenty-three and twenty-seven, while the average number of preceding brother and sister matings which both individuals had in common was slightly less than nine. In three other transplantations in which the number of generations since separation was about twelve, and the number of common generations varied between thirteen and seventeen, the average grade was 5.8. Which of these two factors, the number of generations which donor and host had in common, or the number of separate generations of brother and sister matings in these two individuals is the important one, is impossible to decide on the basis of our data. If the latter factor should play an important part it would be necessary to attribute this effect to possible mutations which occurred during the period of separate breedings: the mutations under these conditions would then have been different in the ancestry of host and donor.

In the large majority of cases in which after nineteen or twenty consecutive brother and sister matings, tissues were transplanted from brother to brother or sister the reactions taking place showed absence of incompatibility, and therefore at least apparent identity between individuality differentials of host and donor. It is therefore probable that under these conditions the difference between the individuality differentials of host and donor disappeared. These two individuals should therefore behave like identical twins. That this condition has actually been reached cannot yet be definitely asserted, inasmuch as it is possible that a slight reaction might still have appeared at a later date. But, on the other hand, we can be certain that in two cases in which such consecutive brother and sister matings had taken place the individuality differentials of brothers had not yet reached identity.

#### SUMMARY

1. The principal results obtained in these investigations are represented in the following list of average grades expressing the reactions of the hosts against transplants. These reactions depend upon the constitution of the individuality differentials of the respective fam-

ilies used for transplantation, and represent the relationship between the differentials.

- Autotransplantation, grade 6.
- Homoiotransplantation, grade 1.6.
- Transplantation within inbred families, grade 5.5.
- Multiple transplantation within inbred families, grade 5.15.
- Successive transplantations within inbred families, grade 4.8 (?) (few experiments).
- Transplantation from brother to brother in non-inbred families: grade 3.35.
- Both combined: {
  - Transplantation from brother to brother in inbred families: grade 5.87 (5.92).
  - 5.84 { Transplantation from brother to brother, hybrids of (5.91) { inbred families: grade 5.78 (5.89).
  - Transplantation from component families to hybrid between inbred families: grade 5.7.
  - Transplantation from hybrid between inbred families to component families: grade 3.24.

2. Within the different inbred families the individuality differentials have reached a very great similarity. The resemblance of the individuality differentials among members of an inbred family which are not closely related is much greater than that among brothers in non-inbred families.

3. There is an identity of the individuality differentials. A complete loss of individuality has not yet been reached within the inbred families. In general the individuality differentials within an inbred family seem to approach more nearly identity the greater the number of generations of brother and sister matings they had in common and the smaller the number of generations since they split off into sidelines of generations of brother and sister matings. On the whole, brothers and sisters seem to have reached identity of individuality differentials after nineteen to twenty generations of continuous brother and sister matings; at least no reaction was elicited in the host on the part of the transplant within the range of time used in our experiments. However, in a few cases there was observed a lack of complete identity of individuality differentials even under these conditions. It is probable that within certain families the homo-



genicity of individuality differentials of the various members of the family is greater than within other families. It is also probable that the average individuality differential of a certain inbred family may differ more from the average individuality differentials of various other inbred families than these differ from each other.

On this basis it would be possible to explain the stronger reactions which tissues of family 13 call forth when they are transplanted into other families, as well as the strong reaction of family 13 serving as host. In addition it is possible that certain strange genes call forth a more intense reaction than other strange genes, or that a host possessing certain genes is able to react more intensely to genes not represented in its own individuality differential.

4. In general the number of strange genes (and perhaps also the intensity of the strangeness of the composing genes) in the individuality differential of the transplanted tissue determines the severity of the reaction of the host against the transplant. The presence of strange genes in the individuality differential of the host; or expressed differently, the absence of certain genes in the transplant or the presence of double genes in the transplant, does not call forth a reaction in the host. On this basis, we can understand the difference between the results of the transplantation from component strain to hybrid, and of the reciprocal transplantation, as compared to the severity of reaction observed after the exchange of tissues within the same inbred family.

#### REFERENCES

1. Loeb, Leo. 1921, *Biol. Bull.*, xl, 143. (Here are found references to the earlier papers of the author and his associates.)  
Loeb, Leo. *Am. J. Path.*, 1926, ii, 99, 301, 315.
2. Wright, Sewall. Bulletin, U. S. Department of Agriculture, Bulletin No. 1090.
3. Little, C. C., and Tyzzer, E. E. *J. Med. Res.*, 1916, xxxiii, 393.
4. Little, C. C., and Johnson, B. W. *Proc. Soc. Exper. Biol. & Med.*, 1921-22, xix, 163.