This is obtained by equating the elements in the first column and first row of the $\mathfrak{M}$-matrix with the corresponding element in its product representation obtained by combining any two commensurate $\mathfrak{M}$-matrices according to the group property.

6. *Ontogenetics.*—At first appearance, the generalized invariant imbedding relation may seem to have no foundation in the established principles of radiative and neutron transport theory. That is, it appears to have been assembled in an ad hoc manner by means of some examples and an intuitively based inductive leap from the classical principles of invariance. However, recent work in the theory of general radiative processes on arbitrary discrete spaces has provided a firm foundation for not only the discrete version of (i) but its most general counterpart in an arbitrary discrete space.[6] Furthermore, the basis for a rigorous derivation in either the discrete or continuous case is given in reference 7.

Thus, while not all details have as yet been worked out, there now exists a complete analytical basis for the development of the concept of invariance, as used in the radiative and neutron transport contexts, from the classical R and T operator level through the invariant imbedding relation level up to the generalized level discussed in this note.

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*SPECIFIC HOMOGRAFT TOLERANCE IN LYMPHOID CELLS OF LONG-LIVED RADIATION CHIMERAS**

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Several lines of experimental evidence, in studies on long-lived radiation bone marrow chimeras, indicate a state of tolerance or altered reactivity to normal tissue homografts,[1–3] heterografts,[4] and transplantable tumors.[5, 6] Long-lived radiation chimeras may be defined as irradiated mice, bearing transplanted cells of genetically foreign bone marrow, which have survived well beyond the usual period of secondary disease.[7] The possible mechanisms involved in the establishment of such homograft tolerance are of evident theoretical and practical interest (cf. ref. 8), particularly in view of the recent formulation of Lederberg,[9] based on the clonal selection

theory of immunity,[10] and in light of the possible relationships among the phenomena of homograft tolerance in radiation chimeras, specific acquired tolerance (Medawar, Billingham, and associates[11]), and tolerance observed under certain circumstances of parabiotic union in mice.[12, 13]

The present report is concerned with delineating further the nature and specificity of the tolerant state of long-lived homologous bone marrow radiation chimeras. In particular, we have directed our attention to the following questions: (1) chimerism of the lymphoid tissue cells of the radiation chimera (are the lymphoid cells of donor origin only, or are both donor and host genotype cells present?); (2) immunological reactivity of lymphoid cells of the chimera; (3) demonstration that lymphoid cells of the chimera are specifically tolerant.

The experimental data to be presented indicate that cells of both donor and recipient genotype are present in the lymphoid tissues of these mice, show that these cells are immunologically reactive, and suggest a state of mutual homograft tolerance between the donor and host cells present.

Materials and Methods.—Radiation chimeras: Young adult male and female (C57L × A)F_1 mice (so-called LAF_1)—3 to 4 months of age—were X-irradiated in a single exposure to 870 r of 250 KVP X-radiation, a dose approximately 100 r above the LD_{100} for these mice. A few hours later they received an intravenous injection of C_3H mouse strain bone marrow cells (5×10^6 nucleated cells) suspended in Tyrode's solution containing penicillin, as described previously.[14] The time course of occurrence of secondary disease and the longevity of such bone-marrow-treated mice have been described elsewhere.[15] In the present experiments, chimeras surviving for a year or more following irradiation and marrow injection were employed as lymphoid tissue donors in the test systems designed to demonstrate the presence of donor type cells (cf. ref. 3) and to study immunological reactivity on the part of these tissues. The C_3H marrow donor strain is known to belong to the histocompatibility antigenic group H-2k; the corresponding alleles in LAF_1 mice are designated H-2a and H-2b.

Isoantigen test system for donor cells: This test for specific isoantigen has been described briefly in a previous report.[3] It is based on the observation[18] that when normal LAF_1 mice receive a single injection of C_3H isoantigen (in the form of spleen cells, for example), a state of immunity is elicited such that protection against lethal X-irradiation given 1 week later is now not afforded by the injection of C_3H marrow.

In the present experiments, the spleen of each chimera was tested for the presence of C_3H isoantigen, essentially as described previously,[3] except that 5 test LAF_1 mice were used for each chimera. The blood of each chimera was tested for the presence of host type (i.e., LAF_1) isoantigen, as follows: 0.2 ml of fresh heparinized blood from the chimera was injected intraperitoneally into normal C_3H mice; one week later, these were exposed to an LD_{100} of X rays (810 r for this strain) and given an intravenous injection of $7–8 \times 10^6$ marrow cells from normal LAF_1 donors. Death of the recipient C_3H mice by 21 days, under these conditions, is interpreted to mean that the chimera mouse blood contains nucleated cells of host (i.e., LAF_1) origin.

Reactivity test system: The test system is based on the previous finding[16, 17] that protection of lethally X-irradiated mice by injected bone marrow is annulled when lymphoid tissue cells homologous to the injected bone marrow are administered. This was interpreted as a homograft rejection of the marrow cells by the injected, immunologically reactive, lymphoid cells, resulting in death of the lethally irradiated recipient mice from bone marrow failure.

Typically, the test system was set up as follows: A group of LAF_1 mice were exposed to a lethal dose of X rays (870 r); they were then divided into 3 subgroups, and each subgroup received (about 1 hr post-irradiation) an intravenous injection of bone marrow cell suspension (5×10^6 nucleated cells per mouse) derived from C_3H, DBA/2, or LAF_1 marrow donors, respectively. Blood was taken from the vena cava of each radiation chimera under chloroform anesthesia and each sample tested for LAF_1 isoantigen. The spleen from each chimera was removed individually, homogenized in a glass Potter-type homogenizer, and the tissue suspension from

each spleen was made up to 20 ml with Tyrode's solution. One ml of this cell suspension was injected intraperitoneally into each of 5 mice in each of 3 irradiated subgroups (above) which had received the bone marrow injections. The spleen cell injections were made approximately 2 hr after the marrow was injected. The remainder of the chimera spleen cell suspension was used in the isoantigen test (above).

Mortality at 21 days was the criterion of effect in this test system. If the chimera lymphoid cells are immunologically competent, they should bring about the rejection of the marrow derived from the third mouse strain (DBA/2), resulting in death of the irradiated recipient mice; furthermore, if the chimera lymphoid cells are tolerant, as is implied from our earlier study showing donor and host-type lung homograft acceptance by such radiation chimeras,[3] then the injected C_3H marrow as well as LAF_1 marrow should not be rejected, resulting therefore in the survival of the recipient, irradiated mice.

General procedures: The mice were put on drinking water containing the antibiotic polymyxin B (1 gm per liter) plus "Biosol" (5 mg per liter) *ad libitum* 1 day prior to radiation exposure and for 7 to 9 days following irradiation as a procedure designed to prevent *Pseudomonas* type infections. They were housed 2 per cage and fed Purina Laboratory Chow.

Results.—Specific immunological reactivity of chimera lymphoid cells: The data on the reactivity test system, employing normal lymphoid cells of known genotype are summarized in Table 1. Injection of 8×10^5 lymph node cells from normal

TABLE 1

REACTIVITY TEST SYSTEM. REJECTION OF BONE MARROW TRANSPLANTS BY HOMOLOGOUS LYMPHOID CELLS

Source of marrow injected (Mouse strain)	Lymph Node Cells Injected		Mortality of irradiated Recipients at 21 days* (No./total)
	Number	Strain	
C_3H	8×10^5	LAF_1	10/10
C_3H	5×10^6	LAF_1	10/10
C_3H	None	—	0/5
LAF_1	1×10^6	C_3H	1/12
LAF_1	5.8×10^6	C_3H	11/12
LAF_1	None	—	1/9

* All recipients are LAF_1 mice.
X-radiation dose: 880 r.

LAF_1 mice caused the rejection of C_3H marrow cell transplants in irradiated LAF_1 mice. When the injected marrow was isologous to the LAF_1 host, a larger number of homologous lymph node cells (in this instance, 5.8×10^6 C_3H node cells) was required to produce this effect.

Turning now to the data in Table 2, it can be seen, first, that in all 8 chimeras

TABLE 2

SPECIFIC REACTIVITY OF CHIMERA LYMPHOID CELLS

Animal no.	Age (months)	Test or Isoantigens*		Reactivity of Chimera Lymphoid Cells versus Marrow Transplants† Mortality (No./total)		
		C_3H (in spleen)	LAF_1 (in blood)	C_3H	LAF_1	DBA/2
1	13 3/4	4/5	Not tested	1/5	0/5	5/5
2	"	5/5	Not tested	0/5	1/5	5/5
3	"	5/5	Not tested	0/5	0/5	5/5
4	"	5/5	Not tested	0/5	0/5	5/5
5	14	5/5	4/4	1/5	0/5	5/5
6	14	5/5	4/4	0/5	0/5	5/5
7	14	4/5	4/5	0/5	0/5	5/5
8	14	5/5	3/3	1/4	0/5	5/5
None‡	—	1/16	5/10	1/9	0/10	3/15

* Transplantation immunity isoantigens; number of mice dead/total. See text.
† Recipients are LAF_1 mice; radiation dose: 870 r. They received I.V. injection of bone marrow from either C_3H, LAF_1, or DBA/2 donors plus I.P. injection of chimera spleen.
‡ Bone marrow injection only.

investigated, the spleens gave positive reactions for the presence of C_3H (i.e., donor) cells, as transplantation immunity isoantigen. In the 4 mice tested, the blood gave a positive test for host type (LAF_1) transplantation immunity isoantigen. The injection of approximately 10×10^6 chimera spleen cells into the lethally X-irradiated LAF_1 mice brought about abrogation of the protective effect of DBA/2 strain bone marrow, a mouse strain carrying the H-2d histocompatibility allele, homologous to both donor and host cells of the chimera. On the other hand, the injection of chimera spleen did not annul the protective effect of either C_3H marrow or LAF_1 marrow. In other words, the chimera spleen cells were reactive against the marrow cells of a third strain (DBA/2), but *not* against bone marrow cells of donor (C_3H) or of host (LAF_1) genotype. The conclusions seem warranted, therefore, that these long-term survivors, following LD_{100} X-radiation exposure and administration of homologus (C_3H strain) bone marrow, are still in the chimeric state after 14 months, that their spleens contain cells capable of immunological reactivity (i.e., rejection of DBA/2 bone marrow cell transplants), and that these spleen cells are at the same time nonreactive, i.e., tolerant, with respect to transplants of C_3H and LAF_1 genotype marrow cells.

Donor type immunologically reactive cells in long-lived radiation chimeras: The next series of experiments were designed to obtain evidence for the presence of immunologically competent cells of donor origin (C_3H genotype) in lymphoid tissues of the chimeras. Two types of designs were used. In the first, ($C_3H \times DBA/2)F_1$ hybrid mice, previously sensitized by injection with LAF_1 isoantigen (as spleen cells) were exposed to a lethal dose of X-radiation (880 r), then given a marrow cell transplant (I.V. injection of 5×10^6 BALB/c marrow cells). These mice then received an injection of spleen cells from the radiation chimeras. The results are summarized in Table 3. Under the conditions of pre-immunization

TABLE 3

REJECTION OF HOMOLOGOUS MARROW CELL TRANSPLANTS BY IMMUNOLOGICALLY REACTIVE CELLS OF DONOR ORIGIN IN SPLEENS OF RADIATION CHIMERAS

Spleen Cells Injected		21-Day mortality of irradiated ($C_3H \times DBA/2)F_1$ recipients* (No./total)
Source	No. of cells	
Chimera No. 13	5×10^6	5/5
Chimera No. 14	5×10^6	5/5
Chimera No. 15	4×10^6	4/5
Chimera No. 16	5×10^6	5/5
Normal C_3H	4×10^6	9/10
Normal LAF_1	5×10^6	1/10

* These recipients had been sensitized (anti-LAF_1) one week prior to X-irradiation (880 r), by a single intraperitoneal injection of LAF_1 spleen cells, as homogenate. A few hours after irradiation, they received a single I.V. injection of 5×10^6 bone marrow cells from normal BALB/c mice, followed by I.P. injection of the spleen cells.

of the ($C_3H \times DBA/2)F_1$ recipient mice (anti-LAF_1), it can be seen that the reactivity of the injected normal LAF_1 spleen cells is prevented, i.e., the BALB/c marrow "takes." By contrast, the injection of normal C_3H spleen cells under these conditions annuls the "take" of BALB/c bone marrow. (This procedure of pre-immunization has been utilized by Siskind *et al.*[28] to prevent or minimize the occurrence of "runt disease" following the injection of homologous lymphoid cells into newborn mice.) Now, when cells from the late chimeras were injected under these conditions, the BALB/c marrow cells were rejected as inferred from the

21-day mortality of the lethally X-irradiated recipients. These results, then, suggest the presence of C_3H type immunologically reactive cells in the spleens of the radiation chimeras.

The second approach to the same question involved the investigation of the capacity of spleen cells from the chimeras to produce the lethal "wasting syndrome" when injected into sublethally irradiated $(C_3H \times DBA/2)F_1$ hybrid mice. If the spleen of the chimeras contain immunologically reactive C_3H cells, then these cells would bear the relationship of parental strain to the $(C_3H \times DBA/2)F_1$ hybrids and evoke this wasting syndrome, as it occurs in other parental-F_1 hybrid combinations.[23] The experimental results are given in Table 4. A lethal syn-

TABLE 4

PRODUCTION OF LETHAL WASTING SYNDROME IN SUBLETHALLY X-IRRADIATED $(C_3H \times DBA/2)F_1$ HYBRIDS BY SPLEEN CELLS FROM RADIATION CHIMERAS

Spleen Cell Injected		21-Day mortality of 500 r X-irradiated $(C_3H \times DBA/2)F_1$ recipients (No./total)
Source	No. of cells	
Chimera No. 13	19×10^6	10/10
Chimera No. 14	18×10^6	10/10
Chimera No. 15	15×10^6	10/10
Chimera No. 16	25×10^6	10/10
Normal C_3H	16×10^6	10/10
Normal LAF_1	20×10^6	0/10

drome with characteristic wasting was elicited in all of the 500 r irradiated $(C_3H \times DBA/2)F_1$ recipients injected with chimera spleen cells. These data show that immunologically reactive C_3H type cells are, indeed, present in the spleens of the 4 long-lived radiation chimeras examined.

Minimal occurrence of secondary disease in irradiated LAF_1 mice injected with C_3H marrow plus chimera spleen: The occurrence of secondary disease is minimal or absent among certain strains of mice when fetal or newborn homologous hematopoietic tissue cells are injected into lethally X-irradiated recipients.[19, 20] This is considered to be a consequence of the fact that fetal or newborn hematopoietic tissues contain immunologically nonreactive and immature lymphoid cell elements, which become tolerant to the isoantigens of the homologous host as they mature. Therefore, no graft-versus-host reaction occurs, and consequently no secondary disease syndrome develops.

In this connection, therefore, it was of interest to ascertain whether injection of spleen cells from the radiation chimeras would modify the occurrence of secondary disease. A group of LAF_1 mice was X-irradiated with 880 r and injected with 3×10^6 C_3H strain marrow cells per mouse. A few hours later, subgroups of 3 to 5 mice from this population received an intraperitoneal injection of spleen cells from each of 8 long-lived radiation chimeras (approximately 14 months post-irradiation). The data are summarized in Table 5. It is evident that the injection of chimera spleen cells, together with normal C_3H bone marrow, greatly reduces the occurrence of secondary disease deaths in this population by 5 months post-radiation. In 4 of the subgroups receiving chimera spleen plus normal C_3H marrow, no deaths occurred, and in the other 4 subgroups, only sporadic late deaths were observed. By contrast, from our previous experience with a large number of lethally X-irradiated LAF_1 mice injected with C_3H marrow, secondary

TABLE 5

MINIMAL OCCURRENCE OF SECONDARY DISEASE IN IRRADIATED LAF_1 MICE RECEIVING C_3H MARROW PLUS CHIMERA SPLEEN

Material Injected		Mortality at 5 Months	
C_3H marrow cells (no.)	Chimera spleen cells (no.)	No./total	Time (days)
3×10^6	11×10^6	0/5	—
3×10^6	12×10^6	0/4	—
3×10^6	16×10^6	1/4	150
3×10^6	5×10^6	1/5	23
3×10^5	17×10^6	1/5	19
3×10^6	16×10^6	1/5	49
3×10^6	11×10^6	0/5	—
3×10^6	13×10^6	0/3	—
None	None	13/13	11.5*

Recipients are LAF_1 mice; radiation dose: 880 r.
* Mean time of death. Range was 9 to 14 days.

disease accounts for deaths in about 60 per cent of the population by 5 months post-irradiation.[15] The injection of normal C_3H lymphoid tissue cells plus C_3H marrow into lethally irradiated LAF_1 mice is known to bring about a more severe and early secondary disease syndrome.[15] Therefore, the fact that in the present experiments the injection of chimera spleen cells greatly reduces the occurrence of secondary disease is again indicative that these cells are nonreactive with respect to the LAF_1 host. Presumably, these tolerant lymphoid cells have repopulated lymphoid sites of the irradiated recipients (cf. ref. 29).

Discussion.—The foregoing experimental data provide additional evidence for existence of a state of homograft tolerance in long-lived homologous radiation chimeras, i.e., those which have escaped or survived the secondary disease syndrome and have lived on for periods of a year or more post-irradiation. The lymphoid cells from these C_3H-LAF_1 radiation chimeras cause the rejection of marrow transplants derived from a mouse strain (DBA/2 or BALB/c) genetically foreign to both the donor and host cell genotype in the chimera itself. However, these chimera lymphoid cells are nonreactive, that is to say, tolerant, with respect to marrow cell transplants of C_3H or LAF_1 origin. These data, taken together with the fact of the presence of C_3H isoantigen, presumed to be C_3H cells (cf. ref. 3), in the lymph nodes and spleen, are tantamount to a demonstration of a state of specific tolerance in these chimeras.

What is the genetic identity of this specifically tolerant, and yet immunologically reactive, population of cells in the lymphoid tissues of these chimeras? The data in Tables 3 and 4 show that at least some of these reactive cells are of donor (i.e., C_3H) origin, derived from the initial bone marrow cell inoculum. The presence of host (i.e., LAF_1) cells also is *inferred* from the fact that the peripheral blood of the chimeras tested gave a positive test for LAF_1 transplantation immunity isoantigens (see Table 2). This could be due to the lymphocytes or to the granulocytes of host origin present in the peripheral blood. Assuming from this that recovery of host lymphopoietic activity has occurred, it may be suggested that a state of mutual tolerance exists between donor and host type lymphoid cells in the long-lived radiation chimeras, in addition to the condition of immunological reactivity in the chimera, leading to rejection of cells of a third mouse strain. Unpublished observations (Davis and Cole) show that these late chimeras will accept tail skin homografts from normal C_3H or LAF_1 donors but regularly reject BALB/c

or DBA/2 skin homograft. However, it should be pointed out in this connection that direct experimental evidence for the presence of immunologically competent cells of host origin in these long-lived chimeras is not yet available.

If the above conclusions be valid, it is pertinent next to consider possible mechanisms by which homograft tolerance may have been produced in these chimeras and the related question of the connection between these findings and that of the phenomenon of acquired tolerance in animals treated with genetically foreign cells at birth.[11] In this context, it has been proposed by Barnes and Loutit[5] and by Barnes *et al.* (1) that skin homograft tolerance in their long-lived radiation chimeras results from "actively acquired tolerance to donor type cells in the sense of Billingham, Brent, and Medawar." [11] Such a view was also put forward by Trentin.[21]

Let us now briefly consider this concept of tolerance in late chimeras in light of the present findings and viewed from the standpoint of the Lederberg formulation.[9] One can readily assume that with supralethal doses of X-radiation (such as used here) all the mature, potential antibody-producing cells, i.e., those not yet sensitized with specific isoantigen, are destroyed. With the administration of genetically foreign isoantigen (in the form of homologous bone marrow cells) immediately after irradiation, we have a situation possibly quite analogous to that of the newborn recipient mouse injected with homologous cells. In the latter instance, there are no mature host cells present, specific for the homologous antigen—cells which are present in the nonirradiated adult animal and which give rise to the immune state. If in time the host lymphoid cells in the radiation chimera can begin to regenerate, they will arise from immature ("hypersensitive") cells in the presence of homologous donor isoantigen, so that any cells potentially specific for that isoantigen will be killed off.[9] This would account for a specific tolerance on the part of the host cells in the chimera, a tolerance which would continue so long as the donor type isoantigen (in this case in the form of dividing cells) persists. In this connection the experimental data of Mitchison[22] and of Nossal[26] further emphasize the necessity of persistence of the foreign antigen for maintenance of tolerance.

An analogous argument to the above would be invoked in the attempt to account for homograft tolerance on the part of *donor cells for host isoantigen* in the late chimera (as proposed here), wherein the original donor cells injected were obtained from mature animals. In this instance, one would have to postulate first that the mature, immunologically competent cells in the marrow inoculum and in the proliferating donor cell population in the irradiated host become functionally inactivated or would die in the course of their continuous and prolonged reactivity against host isoantigen. (It is to be noted that we are speaking here of chimeras which survive the secondary disease syndrome.) Such a concept has been proposed in connection with the parental F_1 hybrid lymphoid cell chimera studies of the authors.[23] In this case, then, inactivation or death of all the mature donor lymphoid cells would lead again to a condition in which only immature cells remain. These would then be subject, in the presence of host isoantigen, to the same clonal selection[9] discussed above for the host cells, resulting in a specifically tolerant cell population of donor origin.

On the basis of these considerations, it may be suggested that a similar state of

tolerance on the part of *donor* cells for host isoantigen exists in the case of actively acquired homograft tolerance following injection of homologous cells into newborn recipients. This view is consistent also with the findings of Rubin[12] and of Martinez *et al.*[13] that prolonged parabiosis between adult parental strain and adult F_1 hybrid mice (both nonirradiated) leads to a state of homograft tolerance on the part of the parental strain mice to F_1 hybrid skin grafts among the surviving parabionts. It is of interest in this context that Simonsen[30] has recently provided evidence suggesting the presence of specifically tolerant donor cells in spleens of mice with runt disease. In this case, runting was produced by the injection of adult C_3H spleen cells into infant ($C_3H \times ST/A)F_1$ hybrids. Thus, development of the state of tolerance in a *nonirradiated, initially adult* lymphoid cell population appears to be possible.

To what extent the present formulation of specific homograft tolerance in long-lived radiation chimeras is critically distinguishable in operational terms from the notion of immunologic tolerance, as a consequence of immunological paralysis in the sense of Felton,[24] we are not as yet in the position to state (cf. ref. 25). However, the observation by Martinez *et al.*[27] that acquired immunological tolerance of skin homografts can be transferred to adult nonirradiated mice by parabiotic union would seem difficult to interpret in terms of immunological paralysis. Experiments aimed at resolving this question further are planned.

Summary.—($L \times A)F_1$ hybrid mice were exposed to an LD_{100} of X-rays (870 r) and were then injected intravenously with bone marrow cells from genetically foreign (i.e., homologous) normal C_3H strain mice. It was found that the long-term survivors (14 months post-irradiation) were still chimeras, as evidenced by the presence in the spleen of transplantation immunity isoantigens specific for the marrow donor strain (C_3H). Immunological reactivity of lymphoid tissue cells from these chimeras was assessed by their capacity, when transferred to lethally X-irradiated ($L \times A)F_1$ mice, to cause the rejection of homologous bone marrow. These chimera lymphoid cells brought about the rejection of marrow transplants derived from normal DBA/2 or BALB/c mice-strains which are genetically foreign with respect to both the donor and host genotype of the chimeras. However, lymphoid cells from the chimeras were found to be nonreactive, i.e., tolerant, towards marrow transplants of ($L \times A)F_1$ or of C_3H origin. Data are presented which indicate strongly the presence of immunologically reactive cells of donor (C_3H) genotype in the lymphoid tissues of these long-lived chimeras. It is suggested that a state of mutual homograft tolerance exists between the donor and host lymphoid cells in these long-lived radiation chimeras, while at the same time, these cells are able to reject grafts of cells which are homologous to both donor and host type. The implications of these findings as they relate to the phenomenon of actively acquired tolerance and to the clonal selection theory of immunity are discussed.

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THE GEOMETRY OF COILING IN GASTROPODS

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The geometrical form of coiled invertebrate shells has long attracted the attentions of zoologists and mathematicians. Coiling is exhibited, to varying degrees, in such diverse groups as the Brachiopoda, Foraminifera, and Mollusca. Most of the work in this field has been directed, however, at the gastropods and coiled cephalopods. Among the early references to the geometry of gastropods are the studies of Réamur,[1] Mosely,[2, 3] Naumann,[4] and Blake.[5] These and other works of the same period have been ably digested and summarized by D'Arcy Thompson.[6]

The principal thesis of Thompson's work is that growth in coiled forms follows