of another kind each contribute six electrons to bonding.

Table 4 presents the ternary configurations. Again the elements and intragroup compounds have been omitted.

Extensive lists of specific periodic compounds, together with $\bar{n}$ and Δx values, compiled from Tables 3 and 4 (*5*) should be of value to anyone contemplating the synthesis of new solid-state materials, whether they be abrasives, thermoelectric substances, semiconductors, or photosensitive compounds. Research workers studying reactions at high pressure should find periodic compounds of interest for a number of reasons: (i) While most of the symmetrical periodic compounds are known, only a very few unsymmetrical compounds have come to light. Although the unsymmetrical far outnumber the symmetrical types, conventional synthesis procedures have apparently failed to disclose many of them. Therefore, it would seem worthwhile to utilize the newly available tool of combined high pressure and temperature in attempting the synthesis of these periodic compounds. (ii) Diamond and diamond-like BN and probably hexagonal B_2O are thermodynamically stable only at high pressure. Consequently, high pressure is required for their synthesis. Many of the proposed periodic compounds will also be stable only at high pressure, but, like diamond, may be retained in a metastable state by reducing the temperature required for synthesis to that of room temperature before the pressure required for synthesis is reduced to normal atmospheric pressure. (iii) Regardless of the necessity of using high pressure for thermodynamic reasons, it is sometimes needed for containing reactants which may be very volatile at the temperature required for synthesis. Present day high-pressure equipment is rapidly becoming a routine tool and can easily contain such substances as sulfur at 1800°C.

Only a small fraction of the total effort in research at high pressure is devoted to chemical synthesis, partly because there have been so few guidelines to point the way to a useful goal. The synthesis of the periodic compounds, particularly of the unsymmetrical variety, should be a new test for the chemist's ingenuity.

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Immunologic Tolerance in Thymectomized, Irradiated Rats Grafted with Thymus from Tolerant Donors

Abstract. *Lewis rats, thymectomized at 5 weeks of age and irradiated at 8 weeks, received grafts of adult thymus and marrow, one or both grafts being derived from donors tolerant to bovine γ-globulin. Challenge 3 or 6 weeks after grafting showed that delayed sensitization could not be induced in animals which received a tolerant thymus or tolerant thymus and marrow, though sensitization to a heterologous antigen (chicken ovalbumin) occurred normally. Arthus reactivity was regained slowly in animals receiving normal thymus and marrow and, to an equal extent, in those receiving grafts from tolerant donors.*

Specific acquired tolerance may be defined as the inability of an individual to respond immunologically to antigens with a particular constellation of determinant groups. It is induced by massive or repeated exposure to antigen in the perinatal period or in the adult rendered nonreactive temporarily by irradiation, treatment with alkylating agents, antimetabolites, or similar substances (*1*). It is also induced in the adult by administration of antigen in a form not readily phagocytized by cells of the reticuloendothelial system, for example, bovine γ-globulin (BGG) from which all aggregated material has been removed by ultracentrifugation (*2*), or by long-continued dosage of antigen (*3*). There is much evidence to suggest that this immunological nonreactivity is related to the persistence of antigen at an undetermined site and that phenomena designated by such terms as tolerance, paralysis, and unresponsiveness may be determined by the same underlying mechanism (*1*). Gowans has shown that tolerance, in two quite different immunologic systems (homograft immunity and antibody formation against sheep erythrocytes), is a property of the recirculating pool of small lymphocytes (*4*). It is not yet known whether this represents the presence of antigenic determinants within these cells at a sensitive site or the absence from the lymphocyte population of clones of cells reactive to the specific determinants. Nor is it clear whether the process that leads to tolerance, which must involve both lymphocytes and antigen, occurs in the peripheral lymphoid organs, in the circulation, or in the source organs where lymphocytes are formed.

We have attempted to assess the role of two possible source organs, the bone marrow and the thymus (*5*, *6*), in the development of tolerance by grafts of these organs from tolerant donors to thymectomized, irradiated recipients that were then challenged with specific antigen at varying intervals. Lewis rats (Microbiological Associates) are made tolerant to BGG by intraperitoneal doses of 20 mg at birth and 50 mg at 4 weeks. They fail to respond at 8 weeks to immunization with BGG, in that they do not develop skin reactivity of the Arthus type (presumably dependent on the presence of circulating antibody) or of the delayed type. In our experiment, normal rats were thymectomized at 5 weeks of age and re-

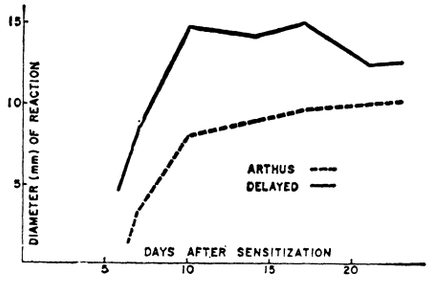

Fig. 1. Course of sensitization in group of six male Lewis rats sensitized by injection of 500 μg of bovine γ-globulin (BGG) in adjuvant into one footpad and skin-tested with 30 μg of BGG at 7, 10, 14, 17, 21, and 23 days. Average values for Arthus reactions (soft edematous lesions, maximal at 4 hours) and delayed reactions (indurated lesions, maximal at 24 hours) are plotted separately.

Table 1. Delayed reactions (24 hours) elicited by skin test with BGG, 10 and 20 days after challenge with BGG and adjuvant.

Treatment group			Average diameter of reaction (mm)*			
			3 week challenge		6 week challenge	
Thymus	Marrow	BGG†	10 days	20 days	10 days	20 days
			Normal animals			
			14.8 (12/12)	13.4 (12/12)		
			Tolerant donors			
			0 (0/60)	0 (0/60)		
			Experimental groups			
Normal	Normal	0	14.5 (9/9)	18.7 (9/9)	12.3 (8/9	13.9 (8/9)
Normal	Tolerant	0	8.5 (5/8)	9.4 (4/7)	9.8 (6/8)	12.1 (7/8)
Tolerant	Normal	0	1.0 (1/9)	1.1 (1/9)	7.3 (5/9)	11.0 (8/9)
Tolerant	Tolerant	0	1.0 (1/10)	1.7 (1/10)	6.9 (6/8)	6.5 (3/8)
Normal	Normal	+	7.0 (4/7)	11.0 (7/7)	9.4 (7/8)	11.9 (8/8)
			Control group, challenged and tested with ovalbumin			
Tolerant	Tolerant	0	16.0 (7/7)	19.4 (7/7)	16.2.(6/7)	17.7 (7/7)

* Figures in parentheses are numbers of animals with positive reactions (diameter greater than 7 mm) in relation to the numbers tested. † BGG, 0.5 to 1.0 mg injected intravenously at time of grafting.

ceived 800 r whole-body irradiation at 8 weeks. Each received immediately a thymus graft from a 10-week-old donor, the thymus having been cut into 4 or 5 pieces and placed subcutaneously in the axilla. At the same time each animal was injected intravenously with 1 to 2 $\times$ 10^8 bone marrow cells. In some recipients one or both of these grafts were from tolerant donors. Three or six weeks after grafting, all recipients were injected in one footpad with 500 μg of BGG in complete adjuvant. They were skin-tested 10 and 20 days later with 30 μg of BGG (*7*) and serum samples were collected for serologic study (*8*). Skin reactions were read at 4 hours (Arthus) and 24 hours (delayed). The course of sensitization in normal rats is shown in Fig. 1.

In rats receiving normal marrow and thymus the ability to develop delayed sensitization was normal 3 weeks after the grafting procedure (Table 1). Rats receiving a tolerant thymus, whether with tolerant or with normal marrow, were completely tolerant at 3 weeks and partially tolerant at 6 weeks, as shown by failure to develop delayed sensitization to BGG. The specificity of this effect was shown by their normal development of reactivity to chicken ovalbumin. Rats receiving a normal thymus and tolerant marrow showed evidence of partial tolerance at the earlier time. Injection of BGG directly into recipients of grafts from normal donors failed to produce tolerance, though there was some diminution of reactivity in animals receiving 500 or 1000 μg of the antigen in this manner. The ability to develop Arthus reactivity was almost completely absent 3 weeks after irradiation and grafting, even in the controls (Table 2). By 6 weeks reactivity could be elicited, though not yet at the normal level, in rats of all experimental groups.

These results suggest that different source organs are perhaps concerned with delayed and Arthus reactivity. The thymus may act as the source of cells taking part in delayed sensitization, as implied by the rapid recovery of this immunologic function in animals receiving a normal thymus and by its failure to appear in animals provided with a tolerant thymus. That the thymus actually contributes lymphocytes to the recirculating pool of these cells and to the lymphocyte population of lymph nodes has been fully established by a number of lines of evidence (see for example *6* and *9*). An unidentified organ of the recipient (spleen?, gastrointestinal lymphoid tissue?) may produce the cells taking part in Arthus sensitization, since this immunologic function was regained slowly after the irradiation injury and since tolerance was not observed, even in animals receiving both thymus and marrow from tolerant donors. Archer *et al.* have suggested that one or more elements of the gastrointestinal lymphoid tissue, notably the appendix and sacculus rotundus, may be analogs of the avian bursa of Fabricius and act as source organs for certain types of antibody formation (*10*). Dietrich and Weigle's recent observation of tolerance in irradiated animals given spleen cells from tolerant donors (*11*) shows that the pool of tolerant cells recirculates through the spleen; but this does not shed light on the problem of the source organ (see also *12*). Tolerance in their experiment was extremely short lived; it disappeared as soon as lymphopoiesis was regained after the irradiation injury.

Tolerance, at least for the delayed type of sensitization, may require entry of injected antigen into the thymus and its persistence there at a critical site. In chimeras tolerant of specific homografts, the thymus may contain up to 24 percent of donor cells, and these may determine the tolerant state (*13*). Nonliving antigens injected systemically actually penetrate the thymus and are found not only in macrophages but in epithelial cells and even in thymic lymphocytes (*14*). There is, however, a clearcut blood-thymus barrier (*15*). In adult animals, Clark has found that, of several antigenic and nonantigenic colloids, only those which were unaggregated and undenatured reached the thymus parenchyma and then only in low concentration (*14*). In newborn animals, on the other hand, larger particles such as thorotrast

Table 2. Arthus reactions (4 hours) elicited by skin test with BGG, 10 and 20 days after challenge with BGG and adjuvant.

Treatment group			Average diameter of reaction (mm)*			
			3 week challenge		6 week challenge	
Thymus	Marrow	BGG†	10 days	20 days	10 days	20 days
			Normal animals			
			8.0 (12/12)	9.3 (12/12)		
			Tolerant donors			
			0 (0/60)	0 (0/60)		
			Experimental groups			
Normal	Normal	0	3.5 (1/9)	3.3 (3/9)	5.5 (4/9)	8.5 (6/9)
Normal	Tolerant	0	1.8 (0/8)	1.1 (1/8)	3.8 (2/8)	7.6 (5/8)
Tolerant	Normal	0	0 (0/9)	2.8 (3/9)	4.9 (4/9)	7.4 (6/9)
Tolerant	Tolerant	0	0.7 (1/10)	2.6 (2/10)	4.0 (1/8)	7.5 (5/8)
Normal	Normal	+	0 (0/7)	6.3 (2/7)	2.5 (0/8)	8.6 (6/8)
			Control group, challenged and tested with ovalbumin			
Tolerant	Tolerant	-0	1.5 (0/7)	14.6 (7/7)	6.5 (4/7)	11.2 (6/7)

* Figures in parentheses are numbers of animals with positive reactions (diameter greater than 7 mm) in relation to the numbers tested. † BGG, 0.5 to 1.0 mg injected intravenously at time of grafting.

may enter the thymus (*16*). Our observations provide a plausible explanation of the known requirement that, to induce tolerance, antigen must be administered systemically in large or repeated doses and in unaggregated form, and that it is most effective in young animals (*1*, *2*). This hypothesis is at least as cogent as that of Eisen and Karush (*17*). These authors suggest that entry of antigen into the reactive cell requires its combination with preformed antibody and that the presence of excess antigen results in formation of complexes which cannot enter the cell. The waning of tolerance must be supposed to depend ultimately on the exhaustion of thymic depots of antigen and initiation of the formation and release of nontolerant lymphocytes. In the tolerant animal, removal of the thymus prevents this transition (*18*), perhaps by simply eliminating the source of nontolerant cells.

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Skin Grafts: Delayed Rejection between Pairs of Cattle Twins Showing Erythrocyte Chimerism

Abstract. *Although dizygotic cattle twins with erythrocyte chimerism exhibit complete tolerance to each other's hematopoietic tissues exchanged* in utero *by way of chorionic anastomoses, they may not be completely tolerant to each other's histocompatibility antigens. Skin grafts exchanged between partners of 21 pairs of chimeric twins were rejected by more than half of the twins in an average of 250 days.*

Most dizygotic cattle twins are tolerant to skin grafts exchanged between members of a pair (*1*). The tolerance results from the reciprocal exchange of cells through vascular anastomoses between the twin embryos. Included in this exchange are primordial hematopoietic tissues so that each one of the twins is a chimera possessing erythrocytes formed by its own tissues as well as those formed by tissues derived (transplanted) from its twin (*2*). Billingham and Lampkin (*3*) reported that most bisexual twins were highly tolerant to their partner's grafts. However, in some pairs there was a transient or persistent chronic inflammatory reaction in one or both of the twins after about 70 days. In two twins, this reaction led to complete rejection of the grafts between 100 to 109 days after grafting. Since the female partners of these twins were freemartins, there was no doubt that the twins were erythrocyte chimeras (*4*).

In experiments to determine the effects of irradiation on erythrocyte chimerism in cattle twins (*5*), we made reciprocal skin transplants between 21 pairs of twins, anticipating that the rejection of these grafts would indicate that tolerance had been abrogated after irradiation (*6*). These experiments have been in progress for more than 2 years, and it is now clear that the grafts are not serving their intended purpose. It is the object of this report to record that more than half of the twins have ultimately rejected their partner's grafts irrespective of irradiation.

The twins were diagnosed as dizygotic on the basis of morphologic differences and blood typing (*7*). Their bloods were subjected to differential hemolysis tests (*8*) to ascertain that they exhibited erythrocyte chimerism. They were all females of dairy breeds, aged from 3 months to 1 year at the time of grafting. Grafts were made essentially according to the technique described (*1*). Pinch grafts from the proximal dorsal side of the ear about 1 cm in diameter were made to the withers of the recipient. Each twin received four autografts, four grafts from its twin (referred to as "co-twin grafts") and two homografts from an unrelated twin. Wherever possible, pigmented skin was transplanted to nonpigmented areas or vice versa. At least two graft beds in each recipient did not receive a skin graft. They were left open to permit an estimate of the healing process and to facilitate accurate readings of graft sites from which grafts were inadvertently lost either by slippage or by adherence to the bandages. The bandages were removed after about 14 days. Readings were made at about weekly intervals for 2 months and monthly thereafter, and survival times were recorded. Histologic examinations of biopsy speci-

Table 1. Fate of skin grafts exchanged between members of pairs of cattle twins with erythrocyte chimerism.

Treatment*	Accept (No.)	Reject† (No.)
Irradiated	11	13
Control	7	10

* Irradiated twins received either 200 to 300 roentgens in a single dose or 450 to 1150 r in a fractionated dose (50 r/week) of whole-body irradiation from Co^{60}. † Mean time for complete rejection was 250 days. Homografts were rejected within 15 days and autografts were accepted indefinitely.

Table 2. Second-set responses of chimeric twins grafted reciprocally.

Twin pairs	Days to complete rejection*	
	1st graft	2nd graft
107†	158	117
108	186	145
117†	326	117
118	‡	§
119†	235	89
120	235	131
125†	‡	§
126	235	131
	Average	
Average	229	121

* Readings made at monthly intervals. † Exposed to 300 roentgens whole-body irradiation from Co^{60}. ‡ No rejection as of 286 days observation. § No rejection as of 180 days additional observation.