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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14 January 1965

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.

mens were made on several grafts to confirm the observation made at the site of the graft.

It is apparent from the data (Table 1) that irradiation did not affect the fate of the co-twin grafts ($P = .6$). However, the salient point of the data is that 56.1 percent (23/41) of the twins rejected their co-twin grafts. In contrast, the homografts were rejected within 15 days, and the autografts were accepted indefinitely. Thus, chimeric twins may not be fully tolerant to each other's skin even though they are tolerant to each other's hematopoietic tissues and thereby sustain erythrocyte chimerism. This situation may be another example of "split tolerance" (*9*) which has been observed among animals made tolerant artificially. Among the 41 twins in the experiment, there were seven whose twins were not studied because of their death or mechanical loss of their grafts. There was considerable variation in the reactions of partners among the 17 remaining pairs. Neither partner of two pairs of twins rejected its twin's grafts during the 200-day period of observation. In contrast, both partners of four pairs of twins rejected their co-twin grafts. Finally, asymmetric responses were observed among 11 pairs; that is, only one member of a pair rejected its twin's graft. There was no apparent correlation between the degree of tolerance exhibited and the asymmetry of the chimeric red-cell populations (*10*).

The time at which complete rejection of the co-twin grafts occurred ranged from 122 to 468 days, and it was not affected by irradiation. The reactions were unlike the acute and decisive homograft rejections observed within 15 days after grafting, but were mild and chronic, lasting from 1 to 3 months. In addition to histologic examination of biopsy tissues, second-set reactions confirmed that the rejections were the result of histocompatibility differences (Table 2). On the average, the second co-twin grafts were rejected in about half the time (121 days) required for the rejection of first grafts (229 days). Twins which failed to reject their first grafts also failed to reject their second grafts.

These data show that varying degrees of tolerance may be established between chimeric twins with respect to histocompatibility antigens. As suggested by Billingham and Lampkin (*3*), this may be due to antigenic differences between the twins and to differences with which the immunologically responsive tissues in each twin became invaded or permeated with cells from its twin.

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Potassium-40 Content as a Basis for the Calculation of Body Cell Mass in Man

Abstract. *On the assumption that the potassium content of the body cell mass is constant it should be possible to estimate body cell mass by measuring potassium-40 activity with a whole-body scintillation counter. Relations of body cell mass to weight, lean body mass, and total body water are demonstrated.*

It was suggested by Forbes *et al.* (*1*) and Anderson and Langham (*2*) that lean body mass might be calculated with the aid of the total potassium content of the body (K). These authors determined K (in milliequivalents) by means of a whole-body radiation counter and divided this value by 68.1 (*1*) or 63 (*2*), respectively, thus obtaining lean body mass in kilograms.

Forbes *et al.* (*1*) have already pointed out that lean body mass in infants and small children cannot be determined in this way, since the potassium content in newborn infants is only 48 meq/kg of lean body mass. Obviously, the change of the content of K in lean body mass during growth is due to a shifting in the relation between intracellular fluid and extracellular fluid, the first of which contains a high concentration of potassium, while the concentration of potassium in the latter is low. It is generally accepted that the relation between intra- and extracellular fluid with growing body size is altered in favor of the intracellular fluid.

Since more than 95 percent of the potassium is contained in the intracellular fluid, it is useful to compare the increase in the amount of the fluid or the growth of body cellular mass (CM), which consists of up to 67 percent of intracellular fluid, with the increase in K. By determining the extracellular fluid in infants and children, and from total body water values I developed the following equation (*3*).

$$CM = 0.42 \times W^{1.11} \qquad (1a)$$

where W represents weight in kilograms. This equation is in good agreement with another developed by Friis-Hansen (*4*), who also found a regression of $W^{1.09}$ for intracellular fluid.

For the calculation of K I made use of 4300 measurements taken by Oberhausen and Onstead (*5*). The data were collected from subjects aged between 6 and 20 years by means of the whole-body radiation counter in Landstuhl (Germany). By correlation of the median values the following equation was obtained for male persons.

$$K = 39.2 \times W^{1.09} \qquad (2)$$

Equation 2 is applicable not only in the range of the measured values but holds true even for fetuses weighing 350 g, as can be seen by a comparison of the values obtained by Iob and Swanson (*7*).

It may be seen from Eqs. 1 and 2 that, during growth, CM and K have an equal relation to W. If a quotient is derived from Eqs. 1 and 2, the result will be the biological K-equivalent (K_{CM}) for 1 kg of cell mass. For man this equivalent is 92.5