Table 3. IgM concentrations in sons of families with four or more sons per family.

| Family number | IgM in serum of sons (mg/100 ml) |
|---|---|
| 04 | 78, 70, 60, 72 |
| 12 | 91, 57, 60, 60, 64, 65 |
| 20 | 60, 64, 70, 56 |
| 23 | 80, 86, 60, 69, 64 |
| 35 | 62, 115, 60, 60 |
| 41 | 50, 88, 93, 59, 65 |
| 48 | 125, 104, 56, 82, 64, 98, 72, 111, 87 |
| 58 | 83, 62, 110, 115, 120 |
| 60 | 60, 80, 31, 49 |
| 62 | 62, 126, 103, 60, 73 |
| 76 | 69, 68, 62, 70 |

similar magnitude. Thus the correlation coefficients between parents and offspring support the hypothesis that the X chromosome carries genes for IgM concentration.

For IgM, the correlation coefficient between sibs (without regard to sex) was .27 for 728 pairs. The correlation coefficients between brothers and those between sisters were of similar magnitude. That is, the fact that sisters carry one X chromosome in common was not reflected in the comparisons. For IgG and IgA concentrations, neither the means nor the correlation coefficients between parents and offspring indicated an effect of the X chromosome.

If the X chromosome does carry genes for IgM concentration, are the genes clustered in a short segment or distributed over the whole chromosome? If the genes are in a short segment, a trend toward two distinct classes of IgM values is expected in the sons of some mothers. In contrast, if the genes are distributed over the whole chromosome no distinct classes are expected. Table 3 shows the IgM values for sons in families with four or more sons. The values in families 12, 35, 41, and 62 reveal a trend toward two classes; those in families 48 and 60 indicate a continuous distribution, and those in the remaining families provide no trend. These data, therefore, do not permit any conclusions about the distribution within the X chromosome of the genes that affect the IgM concentration in man. Obviously, sources other than the X chromosome also contribute to the variation in IgM values.

The higher mean IgM values in females and the relative magnitudes of the correlation coefficients between parents and offspring support the hypothesis that the X chromosome of man carries genes influencing IgM concentration. Rhodes *et al.* (*5*) observed that IgM concentration is influenced by the number of X chromosomes. They found that IgM values were highest in females with three X chromosomes, intermediate in normal females, and lowest in normal males. The IgM values in XO subjects were similar to those in normal males but lower than those in normal females (*6*). The observation that certain types of agammaglobulinemia might be sex-linked (*7*) also points to an association between immunoglobulin and the X chromosome.

If the X chromosome carries a gene or genes for IgM, the higher concentrations in females indicate that for these genes there is no or little inactivation of the second X chromosome. In this respect, these genes resemble the *Xg* locus, which is not subject to inactivation when it is carried on a structurally normal X chromosome (*8*). The increase in IgM concentration when more X chromosomes are present further suggests some form of additive gene action.

Girls are more resistant to certain types of infections than are boys (*9*). The higher concentrations of IgM in girls may explain the difference in susceptibility to infections. However, this difference in susceptibility should disappear in middle-aged individuals because the sex difference in IgM concentrations decreases with increasing age (*3*).

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## Immunologic Tolerance: Role of the Regional Lymph Node

Abstract. *Dansyl chloride, a skin sensitizer when injected in complete Freund's adjuvant, induced marked tolerance and no sensitization when applied to the intact skin of guinea pigs. Application of this chemical to alymphatic skin islands failed to induce tolerance or sensitization. Lymphatic connections between skin and regional lymph nodes were essential for the development of immunologic tolerance.*

Dansyl chloride (dimethylaminonaphthalenesulfonyl chloride) (*1*), a potent skin sensitizer when injected in complete Freund's adjuvant, was found to have the unusual property of inducing marked tolerance and no sensitivity when appled to the skin of guinea pigs. This observation made it possible to study the roles of lymphatic and hematogenous pathways in the induction of immunologic tolerance. Skin islands known to have no lymphatic drainage (*2, 3*) were prepared in 12 guinea pigs (Hartley strain) according to the method of Frey and Wenk (*2*), and 10 $\mu$mole of dansyl chloride in 0.1 ml of acetone was applied within a shaven area (15 to 20 mm$^2$) on the skin of each island. Each of a second group of 20 animals received the same amount of dansyl chloride on shaven intact skin, and each of a third group of 8 animals received 0.1 ml of acetone on intact skin. The sites of application and the skin islands were excised after 7 days. Viability of the islands was confirmed by growth of fur, bleeding when cut, and lack of necrosis. Lack of lymphatic drainage in skin islands prepared by this procedure was verified by the injection of Evans blue dye and India ink particles into the skin of islands, and by subsequent examination of the draining lymph nodes. Four animals in the skin island group and 8 animals receiving dansyl chloride on intact skin were skin-tested 21, 35, and 49 days (*4*) after the initial application of chemical. Twofold serial dilutions of dansyl chloride in acetone beginning with 100

nmole were used as described (*4*). All animals were then injected with 1.0 ml of complete Freund's adjuvant containing 1.0 mg of dansyl chloride and 2.0 mg of *Mycobacterium butyricum*; the dose was divided between the foot pads, inguinal, axillary, and nuchal regions. Skin tests were performed 21, 35, and 49 days after the injections.

The initial application of dansyl chloride to the skin did not result in delayed skin sensitivity in any of the 12 animals tested at each of the three time intervals. When the chemical was injected in complete Freund's adjuvant, all animals receiving dansyl chloride on skin islands and all animals receiving acetone on intact skin developed delayed skin sensitivity on the first skin test. However, in the group receiving dansyl chloride on intact skin, 15 of 20 animals failed to respond on the first skin test to the highest dose of dansyl chloride. Subsequent skin-testing resulted in increased sensitivity of all animals; however by the third skin test, 5 of 20 animals receiving dansyl chloride on intact skin still showed no reaction to the highest test dose (Table 1). Geometric means, with nonreactors assigned a threshold skin reactivity five times the largest test dose, indicated that, by the third skin test, those animals receiving the initial application on intact skin (with both lymphatic and hematogenous drainage) were 45.8 times more tolerant than control animals, while those receiving dansyl chloride on skin islands (lacking lymphatic drainage) were 2.4 times more tolerant than control animals.

These findings suggest that the lymphatic pathway between skin and regional lymph node is important in the development of tolerance to dansyl chloride. The application of this chemical to skin islands with impaired lymphatic drainage resulted in a striking lack of tolerance, while chemical applied to intact skin, with both lymphatic and hematogenous circulation undisturbed, induced a substantial degree of tolerance.

The significance of lymphatic drainage to the regional lymph node has been a subject of debate. Frey and Wenk (*2*) concluded that they were unable to sensitize guinea pigs by applying dinitrochlorbenzene to skin islands, and that the regional node was indispensable in sensitization. Macher and Chase (*5*), in studies with $^{14}C$-labeled dinitrochlorbenzene and picryl chloride injected into the ears of guinea pigs, concluded that the major pathway for escape of these chemicals was the blood stream, and that direct lymphatic drainage was insignificant. By excising the allergenic depot at various time intervals (*6*), they found that a large fraction of chemical escaped by the blood stream during the first 24 hours and concluded that this was associated with the development of tolerance. Our findings suggest that direct drainage to the regional lymph node rather than hematogenous spread is associated with tolerance to dansyl chloride. Other studies have shown significant uptake of labeled dinitrochlorbenzene (*7*) and pentadecylcatechol (*8*) by lymph nodes draining the site of cutaneous application. Some investigators (*9–11*) have demonstrated nearly normal sensitization to dinitrochlorbenzene and pentadecylcatechol when the skin sites were excised during the first 24 hours after application of chemicals, and, in the case of pentadecylcatechol-treated animals, early excision was not associated with greater tolerance.

Table 1. Delayed skin reactivity of guinea pigs after injection of dansyl chloride in complete Freund's adjuvant (third skin test). The skin-test doses of dansyl chloride were administered in acetone.

| Threshold reacting dose (nmole) | Reactors to dansyl chloride (No.) | | Control |
|---|---|---|---|
| | Skin islands | Intact skin | |
| N.R.* | 0 | 5* | 0 |
| 100 | 0 | 6 | 0 |
| 50 | 0 | 2 | 0 |
| 25 | 1 | 3 | 0 |
| 10 | 3 | 1 | 0 |
| 5 | 1 | 2 | 0 |
| 2.5 | 2 | 1 | 4 |
| 1.25 | 4 | 0 | 1 |
| 0.625 | 1 | 0 | 3 |
| Geometric mean reactivity | 3.21 | 62.2 | 1.36 |
| Degree of tolerance† | 2.4 | 45.8 | |

* Some animals failed to react at the highest test dose, and for purposes of calculating the geometric means these animals were assigned a value five times the highest dose. † Ratio of geometric means to geometric mean of control animals.

It is possible that skin islands have altered hematogenous drainage that results in a significantly smaller amount of chemical entering the blood stream. However, the healthy appearance of skin islands, their continued growth of fur, and the histologic examination of their stalks indicate that the circulation in islands is not significantly different from that in normal skin.

The induction of tolerance to dansyl chloride involves lymphatic pathways between the skin and regional lymph nodes. The lack of tolerance in animals receiving dansyl chloride on skin islands suggests that direct drainage to the regional lymph node is essential in developing the degree of tolerance exhibited by animals receiving the chemical on intact skin. While this pathway may be operative in sensitization or tolerance to other simple chemicals, it should be considered that physical and chemical properties of an antigen may influence the routes involved in dispersal from the antigenic depot. It is known that simple chemicals vary greatly in their physical and chemical properties, and that factors such as their reactivity with macromolecules may influence their ability to sensitize or induce tolerance (*4*). The sensitizing and toleragenic properties of these substances may also reflect the pathways of drainage from the antigen depot. Perhaps unique physical and chemical properties not yet defined, as well as pathways of drainage, account for the inability of dansyl chloride to sensitize by the skin route and its failure to induce the competing states of sensitization and tolerance exhibited by other simple chemicals.

Most studies of mechanisms and pathways of delayed skin sensitivity and tolerance have made use of only a few simple chemicals. Studies involving pentadecylcatechol led to a suggestion (*11*) that, during sensitization and tolerance, two pathways may be operative: one involving encounters between blood lymphoid cells and antigen at the allergenic depot with subsequent assisted drainage of the allergen into the lymph node, and another involving a direct lymphatic connection between the skin and regional lymph node. Our study demonstrates that this latter pathway is necessary for the development of tolerance to dansyl chloride. It is anticipated that the role of lymphatic and hematogenous pathways in sensitization and tolerance will be revealed by studying a variety of simple chemicals with diverse physical and chemical properties.

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## "Walking" in the Newborn


Abstract. *Brief daily exercise of the walking and placing reflexes in the newborn leads to a high rate of responding by 8 weeks and to an earlier onset of walking alone. There appears to be a critical period during which the walking response can be transformed intact from a reflexive to an instrumental action.*


If a newborn infant is held under his arms and his bare feet are permitted to touch a flat surface, he will perform well-coordinated walking movements similar to those of an adult. If the dorsa of his feet are drawn against the edge of a flat surface, he will perform placing movements much like those of a kitten. Normally, the walking and placing reflexes disappear by about 8 weeks (*1*). The discovery that they could be preserved intact beyond the second month through active exercise occurred in our pilot investigation with a single infant.

Experiments involving the manipulation of antecedent variables in research on infant mobility have been practically nonexistent; past research is descriptive, defining the various steps in the progression from lifting the head, to sitting, to walking alone (*2*). It has been accepted that this sequence of motor development is invariant, and most tests of infant intelligence are predicated, in large part, on this assumption. Sitting alone momentarily, early stepping movements, and walking alone, for example, are items on the widely used Bayley scale of infant development at 5.3, 7.4, and 11.7 months, respectively (*3*).

Intervention in the motor sequence has been reported with kittens (*4*), but the paradigm is one of deprivation—it shows that restriction of certain activities leads to impairment and not that stimulation can lead to facilitation. In contrast, the present experiment is a test of the generality of the observation that stimulation of the reflexes during the first 8 weeks promotes increased walking and placing.

Twenty-four white, 1-week-old male infants from middle-class and upper-middle-class families were enlisted with the assistance of two local physicians. The birth order of the infants and the ages and socioeconomic status (education and occupation) of the parents were controlled. Six infants each were assigned to the experimental and three control groups.

Infants in the active-exercise group received stimulation of the walking and placing reflexes during four 3-minute sessions each day from the beginning of the second through the end of the eighth week. During each session, the walking reflex was exercised for 2½ minutes and the placing reflex for 30 seconds. Fathers assisted in one of the daily sessions by supporting the infant's knees while the mother held the child erect. Stiffening the knees, it was assumed, would help the infant to learn to support his weight.

Infants in the passive-exercise group received equal amounts of gross motor and social stimulation but without elicitation of the walking and placing reflexes. The infant's legs and arms were gently exercised in a pumping motion while he was lying on his back in his crib or infant seat. When the father was present one of the parents would hold the baby while the other moved his limbs. Infants in the no-exercise group were tested along with the active-exercise and passive-exercise babies at consecutive weekly intervals, but received no special training. A fourth group of infants was tested only once, at 8 weeks of age, to control for the possible facilitative effects of repeated examination.

All training and test sessions were conducted in the infants' homes. The observer explained the program, tested the infant, and demonstrated the training procedure (in the active and passive groups) during the initial visit. With this exception, all training was conducted by the parents, primarily the mother. Each observer tested three infants in each of the four groups. One minute was allowed for testing each of four responses. The observer recorded the number of walking, placing, and straightening (*5*) responses and the age of onset and the frequency of the social smile to the observer when face-to-face at a distance of about 30 cm. An unambiguous walking or placing response with one foot was counted as one, with both feet as two. Only the walking and placing responses resulted in reliable differences among groups.

A strong increase in walking was observed in infants who were allowed to use the walking reflex. The rise to a mean of nearly 30 responses per minute and the low order of responding for all three control groups ($P < .001$) are illustrated in Fig. 1. Individual records revealed that by the end of 8 weeks all six active-exercise infants showed increments in the number of walking responses ranging from 32 to 617 percent over their level at the second week ($P < .01$).

Active-exercise infants also produced more placing responses than any of the control infants ($P < .05$), but there was no consistent increase over base level. Placing in the active group at 8 weeks was similar to the base level (mean = 9.0 responses), whereas infants in the

Table 1. Distribution of ages in infants for walking alone. There is a significant difference among the means [Kruskal-Wallis one-way analysis of variance, $P < .001$ (*11*)].

| | Active-exercise group (months) | Passive-exercise group (months) | No-exercise group (months) | 8-Week control group (months) |
|---|---|---|---|---|
| | 9.00 | 11.00 | 11.50 | 13.25 |
| | 9.50 | 10.00 | 12.00 | 11.50 |
| | 9.75 | 10.00 | 9.00 | 12.00 |
| | 10.00 | 11.75 | 11.50 | 13.50 |
| | 13.00 | 10.50 | 13.25 | 11.50 |
| | 9.50 | 15.00 | 13.00 | |
| Totals | 60.75 | 68.25 | 70.25 | 61.75 |
| Means | 10.12* | 11.38 | 11.71 | 12.35 |

* Infants receiving active exercise walked sooner than infants in the passive-exercise group [Mann-Whitney U, $P < .025$ (*12*)].