of saturated NaCl. After incubation a second pair of 0.5-g samples was examined. In all, 77 dusts were studied.

Live and dead whole mites and insects as well as numerous fragments were found and identified. The live insects were usually recovered from the 1.000-mm sieve where they were found crawling on the larger pieces of dust. Dead mites and fragments were found most commonly in the 0.250- and 0.125-mm sieves. The most common arthropod found was *Dermatophagoides farinae* (Fig. 1). The next most common was *D. pteronyssinus*. Other organisms were (i) migrants such as clover mites (6 samples), (ii) stored-product or nidicolous species such as some species of *Dermatophagoides* itself, or (iii) predators of these species such as mites of the genus *Cheyletus* (9 samples).

Of the 77 samples, 64 were collected from human habitations and 13 were from other sources. Of the dust from nonhuman sources 5 samples were infested with *D. farinae*. Of these samples the largest number of mites found per gram was 34. Of the 64 samples from human sources, 39 contained house-dust mites: 21 had *D. farinae* only, 5 *D. pteronyssinus* only, 11 *D. farinae* and *D. pteronyssinus*, 1 *D. farinae* and *D. evansi*, and 1 *D. pteronyssinus* and *D. chelidonis*. Both *D. evansi* and *D. chelidonis* have been found most commonly in birds' nests. Not only was *D. farinae* found in more samples (33 of 39 positives), but it was also found in greater numbers: up to 69 per gram as opposed to 9 per gram for *D. pteronyssinus*.

The 64 samples of house dust were obtained from Connecticut, New York, New Jersey, Georgia, Washington, California, Nebraska, Wisconsin, and Illinois. No differences were noted in dusts from different geographic areas.

If this survey is truly representative of the mite fauna of dust from which commercial house-dust extracts are prepared, and there is no reason to believe that it is not, one may conclude that more than half (61 percent in this survey) of the extracts used contain extracts of mites of the genus *Dermatophagoides*. If all companies follow the practice of testing each dust for its capability of producing extracts to which patients with clinical house-dust allergy are sensitive, then the number of extracts containing mite extract will exceed 90 percent. Thus, house dust allergy has been, and is now being, detected and treated with extracts of mites of the genus *Dermatophagoides*. This is another example of a case in which specific treatment was developed before the precise cause of the condition was known.

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References and Notes

1. W. F. Mitchell, G. W. Wharton, D. G. Larson, R. Modic, *Ann. Allergy* **27**, 93 (1969).
2. R. Voorhorst, M. I. A. Spieksma-Boezeman, F. T. M. Spieksma, *Allergie Asthma* **10**, 229 (1964); A. Fain, *Acarologia* **9**, 199 (1967); T. Miyamoto, S. Oshina, T. Ishizaki, S. Sato, *J. Allergy* **42**, 14 (1968); K. Maunsell, D. G. Wraith, A. M. Cunnington, *Lancet* **1968-I**, 1267 (1968); R. Voorhorst, *Med. Welt* **5**, 226 (1968).
3. D. G. Larson, W. F. Mitchell, G. W. Wharton, *J. Med. Entomol.* **6**, 295 (1969).
4. The author acknowledges assistance from: contract NIH-69-65 with the Division of Biologics Standards for the dust samples and support; D. E. Johnston and C. A. Triplehorn for identification of mites and insects other than mites of the genus *Dermatophagoides*; and W. C. Lane and the Battelle Memorial Institute for the stereoscan electron micrograph.

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Lymphomas in Mice: Failure of Induction after a Graft-versus-Host Reaction

Abstract. *A mild graft-versus-host reaction induced in (BALB/cJ × DBA/2J)F_1 mice by the administration of parental spleen cells that differ at several weak histocompatibility loci did not influence the development of lymphomas in these animals. Rous sarcoma virus also failed to induce tumors in the runt and control animals. Breast carcinomas, presumably due to contamination of the inoculums with mammary tumor virus, occurred in those experimental groups given parental cells, whether or not they were viable or immunologically competent. We found no evidence that the immunologic process—as represented by the graft-versus-host reaction—is causally related to the induction of neoplasia.*

It has been postulated that the stimulation of immunocompetent cells may be one of the causes of neoplastic proliferation (*1*, *2*). The graft-versus-host disease (GVHD), induced in F_1 hybrid mice by the administration of parental spleen cells, has been considered the ideal system for examination of the possible role of immunologic responses in the induction of cancer in that it provides a genetically defined model of a strong and prolonged antigenic stimulus (*2*). With this system, Schwartz and Beldotti and their co-workers (*3*) and Walford and Hildemann (*4*) found that 50 percent of the mice developed lymphomas after the injection of adult spleen cells into histoincompatible F_1 hybrid hosts. The histoincompatibility was strong (H-2 difference) in the case of Schwartz *et al.* who decreased the usually high mortality of recipient mice by treatment with amethopterine (*3*). The parental spleen cells that Walford and Hildemann injected differed only at the weak H-1 histoincompatibility locus (*4*). In neither study was virus etiology completely ruled out.

That the tumors were of host rather than donor origin (*3*) raised some doubt whether an immunologic reaction caused the induction of these neoplasms. Thus, the question was reexamined in studies on a donor-recipient combination between the extremes represented by mice with a strong difference at the H-2 locus (*3*) and by coisogenic mice that differed only at the weak H-1 locus (*4*).

The strains of mice used, DBA/2J and BALB/cJ (both from Jackson Laboratory), are identical at the H-2 locus. Injection of adult DBA/2J spleen cells into newborn (BALB/cJ × DBA/2J)F_1 hybrid mice (CDF_1) induced a mild, long-term GVHD, due to several weak histocompatibility differences at the H-1 (*5*), H-7 (*6*), and possibly at other, as yet poorly defined, loci.

Cells teased from spleens were gently dispersed in Ringer solution with a fine Pasteur pipette and pushed through a 26-gauge needle to insure single-cell suspension, which was then diluted to contain the desired number of cells in 0.1 ml fluid. The entire procedure was carried out at 4°C. Some portions disrupted by three cycles of freezing and thawing served as control nonviable material. The doses to induce GVHD in newborn CDF_1 mice were four weekly intraperitoneal injections of spleen cells from male DBA/2J mice (4×10^6 cells per gram of body weight). Two groups of mice (groups 11 and 12 of Table 1) received double amounts of parental spleen cells with each injection.

Liver tissue was finely minced, with scissors, in Ringer solution and kept for 5 minutes at room temperature to allow large fragments to settle. The supernatant was decanted and diluted to con-

tain the same numbers of cells as for the spleen cells. Animals treated with amethopterine received five intraperitoneal injections of 0.003 mg per gram of body weight every other day, beginning the day after the fourth injection of parental spleen cells.

To determine whether induced GVHD would break the species barrier to a virus, the experiment was extended by inoculations of Bryan and Schmidt-Ruppin strains of Rous sarcoma virus. These were obtained from Dr. A. Prince. They were partially purified preparations with titers of 1×10^7 focus-forming units (FFU) per milliliter and 3×10^6 FFU per milliliter, respectively. Animals were given 10^5 FFU in 0.1 ml intraperitoneally within 24 hours of birth. Table 1 depicts the design and the results of the experiments.

The majority of mice dying soon after injection of parental spleen cells showed signs of runt disease, most apparent in animals given double amounts of spleen cells (groups 11 and 12). Thus, analyses were limited to animals surviving more than 5 months and maintained to death, or until they were killed at 18 months of age when the experiments were terminated. Tissues of all groups of mice were observed grossly and microscopically.

Among the tumors observed (Table 1), "malignant lymphoma" includes types A and B neoplasms (*7*), of which type A is the most commonly occurring growth of the lymphopoietic tissue. The highest incidence among the inoculated groups was 14 percent (group 3) as compared with the expected 1.67 percent of the 179 control mice (group 1).

In transplantation studies, suspensions of cells from some of the observed lymphomas produced tumors both in adult CDF_1 and DBA/2 mice. This occurred also with one tumor from an untreated mouse. The ease of transplantation may be due to lack of a strong histocompatibility difference between the two parental strains.

Table 1. Incidence of neoplasms in experimental and control groups. The range of day of appearance of the types of tumors appears in parentheses in columns 4 and 6. AMETH, amethopterine; RSV-Br, Bryan strain of Rous sarcoma virus; RSV-S-R, Schmidt-Ruppin strain of RSV.

Treatment group	Percentage of mortality before 150 days of age* (No. of survivors)	Mammary carcinoma (%)	Average day of appearance	Malignant lymphoma (%)	Average day of appearance	Other tumors† (%)
1. Untreated	10.01 (179)			1.67	430 (302–513)	
2. Spleen cells	15.90 (144)	15.99	499 (315–572)	9.03	493 (267–572)	1.39
3. Spleen cells and AMETH	20.80 (85)	20.00	456 (371–519)	14.12	463 (330–574)	1.18
4. RSV-Br	14.00 (63)			4.75	512	6.35
5. RSV-Br + spleen cells	20.77 (107)	28.03	491 (375–581)	6.54	459 (260–567)	2.00
6. RSV-Br + AMETH + spleen cells	27.30 (72)	32.00	472 (360–570)	11.00	416 (208–566)	
7. RSV-Br + AMETH	0.21 (46)	2.17	528	4.35	547 (540–554)	
8. RSV-Br + killed cells	10.10 (34)			3.00	567	3.00
9. RSV-Br + liver cells	10.00 (14)	35.71	494 (404–562)	7.14	542	7.14
10. RSV-S-R	10.20 (41)					
11. RSV-S-R + spleen cells	45.10 (21)	4.80	571	14.28	503 (355–578)	
12. RSV-S-R + AMETH + spleen cells	30.10 (21)	4.80	519	4.80	519	
13. AMETH	6.10 (63)	1.60	343	3.21	545 (543–547)	3.21
14. Killed spleen cells	10.10 (42)	4.78	497 (491–503)	2.39	294	3.58
15. Liver cells	10.20 (20)	5.00	467	15.00	492 (467–512)	

* Indication of runt disease. † Among the tumors were a benign and a malignant hepatoma and others typed as melanoma, hemangioma, leiomyosarcoma, leiomyoma, and carcinoma of the lung. None occurred in any significant percentage.

That adult CDF_1 mice injected at birth with parental spleen cells harbored such elements more than 12 months was shown by Simonsen's discriminant spleen-cell assays (*8*) from four mice with tumors and four mice without tumors. Briefly, 10^7 CDF_1 spleen cells were inoculated intraperitoneally into half of each litter of 8-day-old DBA/2, CDF_1, and BDF_1 (C57B1/6 × DBA/2) mice. The other half of each litter served as uninoculated controls. The mice were killed after 8 days, and weights of body, spleen, and liver were determined. The enlargement of spleen and liver in the cell recipients, with a weight ratio of 1.4 to that of the controls, indicates that immunologically competent cells histoincompatible to the hosts are present in the inoculum.

All eight animals harbored DBA/2 spleen cells since no spleen or liver enlargement was observed in DBA/2 recipients, whereas the test was strongly positive in the BDF_1 and CDF_1 recipients. Only two out of these eight chimeric animals harbored parental cells which had acquired specific immunological tolerance for host antigens; that is, they did not induce any spleen enlargement in CDF_1 mice.

Three of 54 malignant lymphomas in the experiment were type B neoplasms, also called "Hodgkin's-like" lesions of the mouse (*7*). Two of three failed to grow when transplanted into each of the above-mentioned strains of mice. This conforms to other reports (*7*), and strongly contrasts with the behavior of readily transplantable type A neoplasms. The results of the Simonsen assay (*8*) with spleen cell suspensions prepared from these two cases were unexpected. Failure to induce splenic enlargement in DBA/2, CDF_1, and BDF_1 mouse strains indicated that the cells of these neoplasms, derived mainly from organs of the lymphopoietic tissues, were not immunologically competent. Should this finding be confirmed, the nosology and the pathogenesis of these "neoplasms" should probably be reviewed.

The development of mammary tumors in some of the experimental groups but not among the 179 controls (Table 1) was another surprising result. A significant increase was found in those experimental groups which had been injected with parental spleen or liver cells, whether or not they were viable. Several of these CDF_1 mammary tumors were readily transplantable by subcutaneous implants in both DBA/2 and CDF_1 mice, again indicating the

weak histocompatibility difference between the two strains.

It was apparent that the tumors were due to something common to these inoculums, presumably mammary tumor virus which is usually present in the erythrocytes of mice of both sexes that harbor the agent (*9*). Many erythrocytes are present in any spleen or liver preparation, and it appeared likely that mammary tumor virus was carried by the DBA/2J male cell donors. Male DBA/2 mice may harbor mammary tumor virus (*10*), as indicated by studies at the Jackson Laboratory.

In conclusion, graft-versus-host disease that results from the inoculation of parental spleen cells differing at more than one histocompatibility locus failed to induce a significant number of malignant lymphomas in the recipient mice. Neither were tumors observed in the mice inoculated with Rous sarcoma virus, possibly because partially purified preparations were used rather than crude extracts (*11*). This suggests that factors other than GVHD itself (such as mammary tumor virus in the present study) may have been involved in those experiments in which malignant lymphomas occurred (*3, 4*). The relation of immunologic phenomena to neoplastic proliferation remains to be clarified.

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References and Notes

1. W. Dameshek and R. S. Schwartz, *Blood* **14**, 1151 (1959); D. Metcalf, *Brit. J. Cancer* **15**, 769 (1961).
2. A. Tyler, *J. Nat. Cancer Inst.* **25**, 1197 (1960); *Biological Interactions in Normal and Neoplastic Growth* (Little, Brown, Boston, 1962); H. S. Kaplan and D. W. Smithers, *Lancet* **1959-II**, 1 (1959); I. Green, M. Inkelas, L. Allen, *ibid.* **1960-I**, 30 (1960).
3. R. S. Schwartz and L. Beldotti, *Science* **149**, 1511 (1965); R. S. Schwartz, J. Andre-Schwartz, M. Y. K. Armstrong, L. Beldotti, *Ann. N.Y. Acad. Sci.* **129**, 804 (1966); J. Andre-Schwartz, R. S. Schwartz, L. Mirtl, L. Beldotti, *Amer. J. Pathol.* **50**, 707 (1967); M. Y. K. Armstrong, R. S. Schwartz, L. Beldotti, *Transplantation* **6**, 1380 (1967).
4. R. L. Walford and W. H. Hildemann, *Amer. J. Pathol.* **47**, 713 (1965); R. L. Walford, *Science* **152**, 78 (1966).
5. R. J. Graff and G. D. Snell, *Transplantation* **6**, 598 (1968).
6. G. D. Snell and J. H. Stimpfling, *Biology of the Laboratory Mouse*, E. L. Green, Ed. (McGraw-Hill, New York, 1966), chap. 24, p. 457.
7. T. B. Dunn and M. K. Deringer, *J. Nat. Cancer Inst.* **40**, 771 (1968).
8. M. Simonsen, J. Engelbreth-Holm, E. Jensen, H. Poulsen, *Ann. N.Y. Acad. Sci.* **73**, 834 (1958).
9. S. Nandi, D. Knox, K. B. DeOme, M. Handin, V. V. Finster, P. B. Pickett, *J. Nat. Cancer Inst.* **36**, 809 (1966); S. Nandi, M. Handin, L. Young, *ibid.*, p. 803; S. Nandi, *Twentieth Annual Symposium on Fundamental Cancer Research* (Univ. of Texas Press, Houston, 1966); ———, K. DeOme, M. Handin, *J. Nat. Cancer Inst.* **35**, 309 (1965).
10. J. Dory, the Jackson Laboratory, Bar Harbor, Maine, personal communication.
11. R. Bather, A. Leonard, J. Yang, *J. Nat. Cancer Inst.* **40**, 551 (1968); J. Mark, *Int. J. Cancer* **3**, 663 (1968).
12. Supported by NCI grant CA 10,000, NYCHRC grant U-1840, and the Leukemia Society. G. B. Rossi was a Fellow of the Leukemia Society of America, Inc., while on leave of absence from the Istituto di Anatomia ed Istologia Patologica, Naples University School of Medicine, Naples, Italy. We thank C. Wong, H. Barromi, and G. Holland for technical assistance.

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Mosaic Ruler

Mills's idea (*1*) for a "more economical" version of my (*2*) hypothetical mosaic unit ruler has come to my attention. He qualifies his suggestion by either omitting unit 21.6 cm, or incorporating it differently from the other units. However, I find so many (Fig. 1) classical floor-mosaic patterns this size compared with the other mosaic unit sizes that I regard it probable that, at least from the mosaicists' point of view (*3*), unit 21.6 cm was as basic as the others, and I would expect it to appear like the others on their rulers.

However, while Mill's ruler is simpler in the sense of having fewer calibrations, having made one, I find it much trickier to use than mine which is simply marked with each unit in turn from a zero at one end. The latter arrangement happens to coincide with that usually found on other ancient rulers.

This problem may come to be resolved, for, following Ledin's comment (*4*), a picture (*5*) has come to light leading to the possibility (*6*) that an original mosaicist's ruler may be contained in a burial in the Catacombs of Priscilla at Rome.

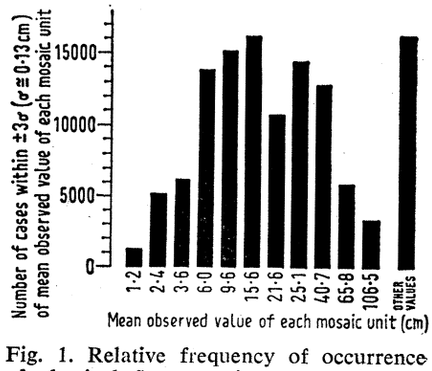

Fig. 1. Relative frequency of occurrence of classical floor-mosaic pattern sizes in sample of 121,265 observations.

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References and Notes

1. R. L. Mills, *Science* **162**, 1306 (1968).
2. R. E. M. Moore, *ibid.* **161**, 1358 (1968).
3. Supported by alignment intervals occurring about as often at 21.6 cm as at the other mosaic unit intervals; see R E. M. Moore, *Nature* **217**, 482 (1968).
4. G. Ledin, *Science* **163**, 704 (1969).
5. A. Bosio, *Roma Sotterranea* (Rome, 1632), vol. 3, p. 505.
6. R. E. M. Moore, *Fibonacci Quarterly*, in press.

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Dorsal Root Potentials Produced by Stimulation of Fine Afferents

Concerning the reports (*1–3*) that volleys in afferent unmyelinated fibers produce a negative dorsal root potential (DRP) in contrast to an earlier finding (*4*) such that impulses in fine afferents were said to produce a positive DRP, Zimmerman (*1*) says that his finding abolishes "one of the basic postulates of a recent pain theory" (*5*) and Vyklicky *et al.* (*3*) state that their results deny "a basic tenet" of the theory. The paper to which they refer proposed no more than that the input-output relations of hypothetical dorsal horn cells were modulated by what was termed a "gate control mechanism." Impulses arriving in certain fine afferent fibers tended to open the gate by facilitation, while certain large fibers closed it by inhibition. A possible presynaptic mechanism was discussed, but there was doubt as to whether the mechanism of the modulation was presynaptic, postsynaptic, or both. To emphasize this uncertainty, the diagram of the gate control mechanism showed a box around both pre- and postsynaptic structures. The location of the facilitating mechanism was never a "basic postulate," let alone a "tenet." The theory does require that some modulating mechanism should exist but does not specify its location. Evidence continues to accumulate that a modulating mechanism does exist. For example, lamina 5 cells and flexor motoneurons are facilitated by some fine afferents and inhibited by some large afferents (*2, 6*). Irrespective of the sign of DRP's, we have still to face the exist-