is important for accurate counting of young worms. Larvae develop in blood vessels deep within the brain; at about 3 weeks they appear on the surface, leave the blood vessels, and, in heavy infections, produce extensive surface hemorrhage that is usually the immediate cause of death. With lighter infections, tolerated by the rodent host, larvae migrate from the brain to the lungs after about 18 days.

The epidemiology of eosinophilic meningoencephalitis suggests that direct ingestion of infected snails or slugs is unlikely to account for the majority of cases (*1*). Transport hosts presumably are infected by eating infected mollusks, either living or decomposed.

Our evidence of infection by ingestion of raw lettuce or of vegetables on which infected slugs had fed represents a new approach that may help to explain infection without ingestion of the primary host. However, the phenomenon of spontaneous shedding of larvae may be restricted to heavily infected small slugs of the *Microparmarion* type found in Malaya. No evidence of natural shedding is available from the heavier-bodied true slugs or from hard-shelled land snails or pulmonate hosts. Although larvae do leave aquatic snails, they usually do so when the hosts are dead or dying; this process is probably not epidemiologically important.

Samples of lettuce, purchased from Kuala Lumpur public markets, showed small numbers of living, infective third-stage larvae of *A. cantonensis*; they averaged 2 to 3 larvae per 50 g of leaf. Identification of larvae was verified by their successful development in brains and lungs of white rats.

Whereas human eosinophilic meningoencephalitis attributed to *A. cantonensis* is common in Thailand and elsewhere in the Pacific basin (*2*), it has never been observed in Malaysia—possibly because of failure in detection. *Microparmarion,* however, is not apparently a significant host outside Malaysia; in Thailand the disease is thought to be caused by ingestion of raw, infected, aquatic snails (*Pila* spp.) (*3*).

Previous studies in our laboratory (*4*) demonstrated that laboratory-reared rats, infected for the first time with small numbers of *Angiostrongylus*, resist subsequent infection to a considerable degree. Three exposures to 10 or 15 larvae will protect white rats against 1000 larvae, which are ordinarily lethal to controls within 25 to 30 days. Man's unwitting exposure by way of contaminated lettuce raises the interesting conjecture that he may become actively immunized against eosinophilic meningoencephalitis in areas in which this slug is the predominant intermediate host.

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Chromosome Abnormality in Rat Leukemia Induced by 7,12-Dimethylbenz[a]anthracene

Abstract. *A high percentage of consistent chromosome abnormality, trisomy of the longest telocentric chromosome, was found in leukemias induced in rats of the Long-Evans strain by pulse doses of 7,12-dimethylbenz*[a]*anthracene. Cells with this abnormality were large, immature, and mononuclear and tended toward erythroblastic maturation.*

Since the first report (*1*) on the presence of a consistent chromosome abnormality (Ph^1 chromosome) in human chronic granulocytic leukemia, there have been efforts to find other consistent chromosome abnormalities in tumors, in either humans or experimental animals. However, there have been few positive results (*2*). We now report on a consistent chromosome abnormality found in the cells of rats with leukemia induced by 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA). This abnormality consists in the trisomy of the longest telocentric chromosome.

We used random-bred male and female rats of Long-Evans strain, originally separated from the colony in the Ben May Laboratory for Cancer Research, University of Chicago. The injection of 7,12-DMBA was started on day 27 after birth. Each animal received four to six intravenous injections of 7,12-DMBA fat emulsion (*3*) (Fig. 1). The pulse injections of 7,12-DMBA induced many leukemias within 120 days after the first injection. Chromosome preparations of femur bone marrow and spleen (*4*) were made immediately after the leukemic rats were anesthetized and killed at the terminal stage by puncture of the aorta. Counting of chromosome num-

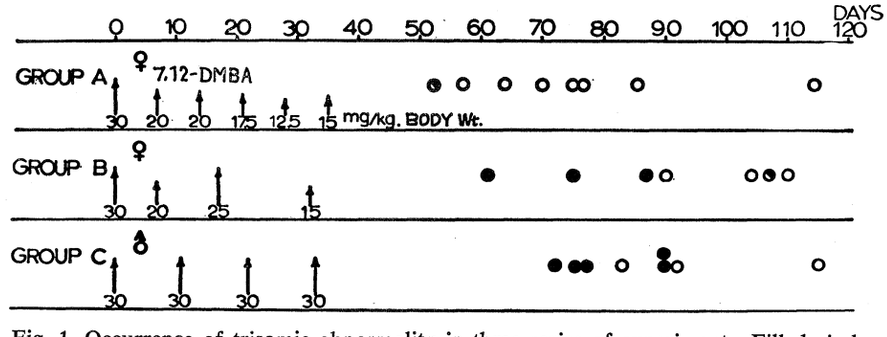

Fig. 1. Occurrence of trisomic abnormality in three series of experiments. Filled circles, cases with trisomic chromosome abnormality; open circles, cases with no trisomic change; half-filled circles, cases having a low percentage of trisomy. In group A, of 80 percent that developed leukemia within 120 days, only one case (12.5 percent) had the trisome. In group B, incidence of leukemia was 58.3 percent, and of trisomy it was 57.1 percent. In group C, incidence of leukemia was 72.7 percent, and of trisomy it was 62.5 percent.

bers and chromosome analysis of leukemia cells were performed in about 50 metaphases for each specimen. Where there was no visible chromosome abnormality in 50 metaphases, an additional 50 were analyzed to confirm the absence of abnormal chromosomes. Five randomly chosen, normal rats of the same strain were analyzed for normal chromosome pattern. The modal chromosome number was 42, and no chromosome abnormality was detected. The chromosomes were classified into three groups in accordance with their centromere position: meta- and submetacentrics (A), subtelocentrics (B), and telocentrics (C) (Fig. 2).

The chromosome abnormality most frequently observed in bone marrow and spleen cells of leukemia rats was trisomy in the C-1 chromosome. Among 23 leukemia rats examined, ten (43.3 percent) had this type of chromosome abnormality. Although the typical C-1 trisomy (Fig. 3) was predominant, rats 3 and 5 had a leukemia cell line characterized by the presence of one C-1 and one long metacentric marker chromosome probably composed of two C-1 chromosomes fused together. Therefore, it was evident that these cells were essentially trisomic for C-1 chromosome (Fig. 4). In rat 8, five different stemlines were found at the same time. There were fewer cells with trisomic change in rats 1 and 5, and these did not constitute the stemline. In general, there was no striking difference between bone marrow and spleen as to the percentage of cells with C-1 trisomy.

Four anatomical types of leukemia were induced by injection of 7,12-DMBA (*5*). Except for case 3 in which lymphocytic leukemia was also present, all the leukemias that we examined belonged to the hepatic type (*5*), the predominant type of rat leukemia induced by 7,12-DMBA and characterized by a huge red liver due to marked proliferation of leukemia cells in hepatic sinusoids. The cells of this type were too complicated to be classified into simple hematological categories. In the blood of the trisomic cases, there was an increased number of large, atypical mononuclear cells with a round nucleus of dense chromatin and with a strong basophilic cytoplasm. The transition of these cells to basophilic erythroblasts and nucleated red cells was also evident. Fresh whole blood (0.2 ml) taken from three trisomy-positive leukemic rats was injected intraperitoneally in newborn rats; 3 to 5 weeks later, the rats with injections developed leukemia characterized by hepatosplenomegaly, peritoneal implantation, and a striking increase of blood leukocytes. All these cells had trisomic C-1 chromosomes and resembled cells of the erythroblast series corresponding to the donor cells. Hence, it seemed plausible to conclude that the erythroblastic leukemia cells had trisomic C-1 chromosome. Blood from rats without this chromosome abnormality contained a much lower percentage of erythroid cells. There was a different incidence of trisomic leukemias among the experimental groups (Fig. 1), possibly due to the difference in the interval of the injections.

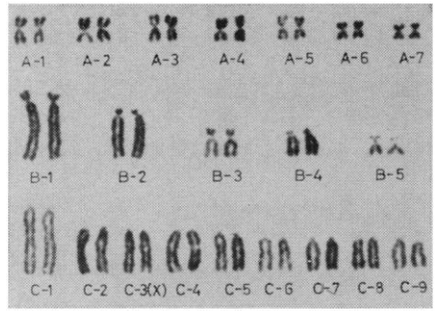

Fig. 2. Idiogram of a normal female Long-Evans rat.

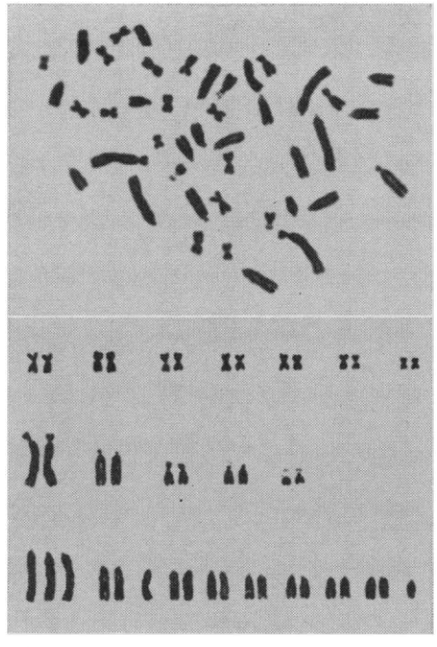

Fig. 3. Metaphase plate and karyotype with typical C-1 trisomy (case 9).

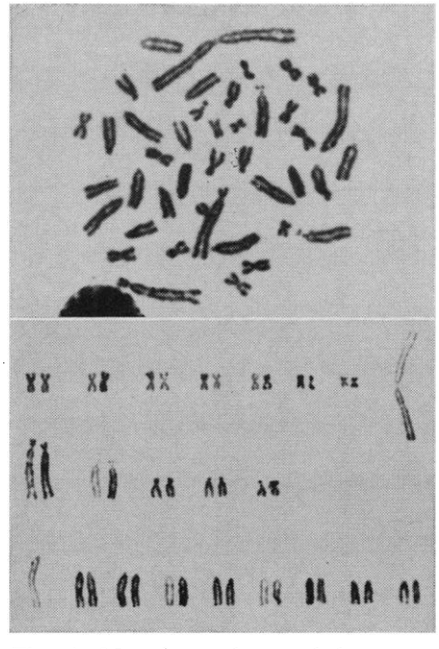

Fig. 4. Metaphase plate and karyotype with C-1 trisomy of translocation type (case 3).

One case (in the Wistar strain) with a similar trisomy in rat leukemia induced by 7,12-DMBA was recorded (*6*). It had the same idiogram as our case 10. The C-1 chromosome was frequently and significantly involved in leukemias induced by 7,12-DMBA. Whether the anomaly is of primary importance in leukemogenesis is still unknown. However, in view of the fact that it is limited to one leukemia cell type, it may be as significant as the Ph^1-chromosome in human leukemia. The presence of several stemlines in some cases revealed the mosaic nature of chemically induced leukemia and raised new questions regarding the origin and development of leukemia cells. It is of importance to explain the trisomic abnormality from cytogenetical and hematological aspects.

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