tion starch-gel electrophoresis (*5*) of the globin, prepared according to Beale (*6*)], indicated that the anomaly is in the beta chain (*7*). Preliminary studies of peptide mapping on tryptic digests of the globin have revealed that the β-T-3 peptide is abnormal in the present G hemoglobin; the β-T-3 section includes the 13 amino acid residues from position beta-18 to beta-30. The name G Taegu has been adopted pending further work.

It appears significant that no instances of hemoglobin E were found among these Korean subjects. Hemoglobin E has been found among numerous ethnic groups of Southeast Asia (*8, 9*); in fact, it appears to have been found in almost every group examined in that part of the world. In most of those groups the incidence of hemoglobin E is at least 1 percent and in some groups, such as the Thai, Burmese, and Cambodians, it is quite high. In the Chinese groups studied the incidence is appreciable but lower; for example, Vella reported 0.27 percent among 10,031 Chinese subjects in Singapore and surrounding areas (*10*), and 0.09 percent were found among normal Chinese residents in Taiwan (*11*). This trend of lower incidence in the Chinese becomes more marked in the Japanese, in which one subject with hemoglobins A and E was found among approximately 120,000 Japanese subjects tested (*12*). Similarly, among the aborigines in Taiwan, who are of Proto-Malayan stock, but relatively long-time inhabitants of Taiwan, no hemoglobin E was found among 4501 subjects (*9*). The present results of no hemoglobin E among 6700 Koreans fit the same pattern of reduced incidence of hemoglobin E in northern and eastern Asia.

R. QUENTIN BLACKWELL
JEANETTE TUNG-HSIANG HUANG
Department of Biochemistry, U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan, Republic of China
IHL HYEOB RO
Department of Pharmacology, Sookmyung Women's University, Seoul, Republic of Korea

References and Notes

1. R. Q. Blackwell, I. H. Ro, J. T.-H. Huang, L.-C. Chien, *J. Formosan Med. Ass.* (abstract), in press.
2. R. Q. Blackwell and J. T.-H. Huang, *Clin. Chem.* **11**, 628 (1965).
3. O. Smithies, *Biochem. J.* **71**, 585 (1959).
4. C. A. J. Goldberg, *Clin. Chem.* **4**, 484 (1958); T. Aronsson and A. Grönwall, *Scand. J. Clin. Lab. Invest.* **9**, 338 (1957). Forty samples were run on each starch-gel slab with approximately 2 μl of hemolysate used for each test. Electrophoresis was run for approximately 16 hours at 100 volts 3) volt/cm); total current in each slab varied from 4 to 7 ma.
5. A. I. Chernoff and N. M. Pettit, Jr., *Blood* **24**, 750 (1964).
6. D. Beale, in H. Lehmann and R. G. Huntsman, *Man's Haemoglobins* (North-Holland, Amsterdam, 1966), chapt. 25, p. 300.
7. Analyses performed in this laboratory by C. C. Wang.
8. Lie-Injo Luan Eng, *Ann. Human Genet. London* **28**, 101 (1964).
9. R. Q. Blackwell, J. T.-H. Huang, L.-C. Chien, *Human Biol.* **37**, 343 (1965).
10. F. Vella, *Acta Haematol.* **23**, 393 (1960).
11. R. Q. Blackwell, J. T.-H. Huang, L.-C. Chien, *Israel J. Med. Sci.* **1**, 759 (1965).
12. S. Shibata, I. Iuchi, H. B. Hamilton, *Proc. Japan Acad.* **40**, 846 (1964).
13. We thank Professor Kum Haw, M.P.H., former Dean of the College of Pharmacy, Sookmyung Women's University, Dr. Tae Lim Yun, President, Sookmyung Women's University, Seoul, and other Korean officials for their aid in the study and Misses Gloria Ku, Elsa Pan, Ling Yen, Li-Chin Chien, and Grace S.-J. Chen for technical assistance. Messrs. J. V. Davenport, HMCS, USN, and P. V. Aspacio, HM1, USN, provided valuable support in expediting the field collections and shipments of specimens. This work was accomplished under the Bureau of Medicine and Surgery work unit MR005.20.0099. The study was funded in part under U.S. Public Law 480, Section 104(c) and in part by the Bureau of Medicine and Surgery, Department of the Navy, Washington, D.C.

22 August 1967

Angiostrongylus cantonensis: Proof of Direct Transmission with Its Epidemiological Implications

Abstract. *Infective larvae of the rat lungworm* Angiostrongylus cantonensis, *presumed cause of human eosinophilic meningoencephalitis, are shed in mucus exuded by naturally infected Malayan slugs* (Microparmarion malayanus). *Larvae passed by slug hosts were recovered from lettuce and produced normal infection in white rats. Lettuce sold in the local public market also yielded small numbers of infective larvae. Experimental evidence from rats suggests that the local human population, exposed to repeated low-level infections, may become immunized against the rare massive exposure and against clinical disease that might otherwise result after ingestion of heavily infected raw mollusks.*

Infection of rats with third-stage larvae of *Angiostrongylus cantonensis* can occur without ingestion of the mollusk intermediate host. In Malaya the commonest intermediate host is *Microparmarion malayanus,* an abundant nocturnal shelled slug of field and garden. Normally 40 to 50 percent of the slugs from our collection locality are infected, harboring on average 250 larvae; levels of infection range from 80 to 4000 worms per infected slug. Infective larvae pass spontaneously from this mollusk while it is feeding; embedded in the mucus trail left by the slugs, they remain viable for at least 72 hours. In our initial experiments we placed 50 field-caught, naturally infected slugs on five lettuce leaves (about 50 g) for 24 hours. After removing the slugs, we washed the leaves and searched them for *Angiostrongylus* larvae. Worms recovered from the washings were examined for removal of the abundant free-living worms as well as parasitic nematodes other than *Angiostrongylus.* From 30 such 50-g batches of lettuce, 368 living third-stage larvae of *Angiostrongylus* were recovered, averaging about 0.25 larva shed per slug (0.62 per infected slug on the average) and 2.5 larvae per individual leaf per night (or 12.5 larvae per batch of 5 leaves). These data include only larvae removed and identified; undoubtedly others adhered to the leaves or were missed.

Larvae recovered from the lettuce were fed by pipette to unanesthetized white rats. Fourth-stage larvae and subadult *Angiostrongylus* were found in the brains of these rats 13 to 15 days later. Adult worms were recovered from the lungs and hearts of other rats after 34 to 36 days (Table 1).

Because of difficulty in location of all worms passed from the slugs, exposed lettuce was also fed to rats. Fewer adults but more larvae were recovered after such feedings than after direct ingestion of larvae, but the numbers were small and no conclusions can yet be drawn. The time of autopsy

Table 1. Worms recovered from ten white rats infected with *A. cantonensis* larvae collected from lettuce.

Larvae fed (No.)	Days before killing	Worms recovered (No.)
28	15	1
47	15	3
28	13	0
44	34	13
45	34	10
31	33	4
28	36	1
30	36	5
42	35	20
45	34	12

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.

DONALD HEYNEMAN*
BOO-LIAT LIM
International Center for Medical Research and Training (Hooper Foundation), Institute for Medical Research, Kuala Lumpur, Malaysia

References and Notes

1. G. D. Wallace and L. Rosen, *Amer. J. Epidemiol.* **81**, 52 (1965); L. Rosen, G. Loison, J. Laigret, G. D. Wallace, *ibid.*, in press; J. E. Alicata and R. W. Brown, *Can. J. Zool.* **40**, 755 (1962); J. E. Alicata, *South Pacific Comm. Tech. Paper 139* (1963); ——— and D. D. McCarthy, *Can. J. Zool.* **42**, 605 (1964); J. E. Alicata, *ibid.* **43**, 667 (1965).
2. R. Franco, S. Bories, B. Couzin, *Med. Trop.* **20**, 41 (1960); S. Punyagupta, *Amer. J. Trop. Med. Hyg.* **14**, 370 (1965); P. Ketsuwan and A. Pradatsundarasav, *ibid.* **15**, 50 (1966); S. R. Horio and J. E. Alicata, *Hawaii Med. J.* **21**, 139 (1961); L. Rosen *et. al., J. Amer. Med. Assoc.* **179**, 620 (1962); J. E. Alicata, *Can. J. Zool.* **40**, 5 (1962); K. Jindrak and J. E. Alicata, *Ann. Trop. Med. Parasitol.* **59**, 294 (1965); D. F. B. Chav and L. Rosen, in press.
3. S. Punyagupta, *Amer. J. Trop. Med. Hyg.* **14**, 370 (1965).
4. D. Heyneman and B.-L. Lim, *Med. J. Malaya* **20**, 154 (1965).
5. We thank Leon Rosen for permission to examine manuscripts currently in press.
6. Aided by the Hooper Foundation, San Francisco School of Medicine, University of California, from NIH research grant TW 00144.
* Present address: Hooper Foundation, San Francisco Medical Center, University of California, San Francisco 94122.

18 October 1967

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-

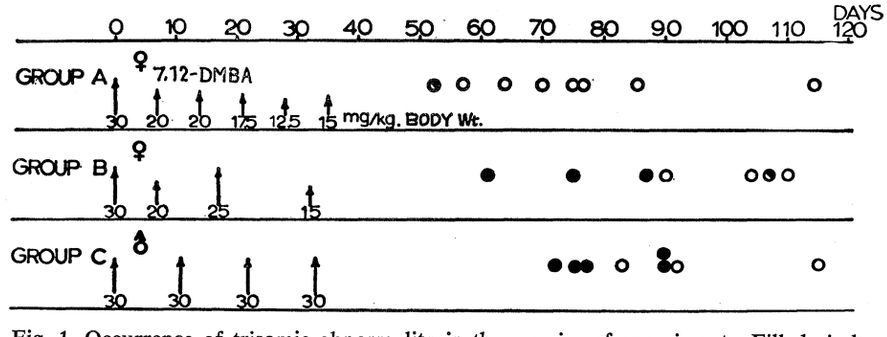

Fig. 1. Occurrence of trisomic abnormality in three series of experiments. Filled circles, cases with trisomic chromosome abnormality; open circles, cases with no trisomic change; half-filled circles, cases having a low percentage of trisomy. In group A, of 80 percent that developed leukemia within 120 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.