Reports

Inherited Electrophoretic Hemoglobin Patterns among 20 Inbred Strains of Mice

Abstract. The hemoglobin from mice of six inbred strains is of the single-spot electrophoretic type, and that from 14 inbred strains is of the diffuse type. No selective advantage is apparent for either type. The distribution among strains shows some relation to the history of the development of the strains.

Recent studies by Ranney and Waelsch (*1*) have demonstrated two distinct types of hemoglobin in normal healthy mice from four standard inbred strains and from seven special mutant strains. They have also shown that the difference between animals with single-type hemoglobin, giving a single homogeneous spot on filter-paper electrophoresis, and those with diffuse-type hemoglobin is due to a single genic substitution (*2, 2a*). In the present investigation, the hemoglobin patterns of 8 to 10 mice from each of 20 different inbred strains were determined by one of us (P.S.G.) by means of a modification (*3*) of the starch block electrophoretic technique developed by Kunkel *et al.* (*4*). The mice were young adults (6 to 9 weeks old) of both sexes, from the Inbred Nucleus of the Jackson Laboratory. All of the mice tested from any one inbred strain showed the same hemoglobin type (*5*).

Mice from six inbred strains (C57BL/6, C57BR/cd, C57L/He, C58, SWR, and WK) carried single-type hemoglobin, and mice from 14 inbred strains (A/He, A/Jax, AKR, BALB/c, C3H/Jax, C3HeB, DBA/1, DBA/2, MA/My, RFM, 129, WB, WC, and WH) carried diffuse-type hemoglobin (*2a*).

The mean erythrocyte number and size characterizing young adults of ten of these inbred strains has been established by previous investigations (*6*). Three single-type strains (C57BL/6, C57BR/cd, and C57L/He) gave mean erythrocyte values of 9.66 to 10.54×10^6 cells per cubic millimeter, while seven diffuse-type strains (A/He, A/Jax, AKR, BALB/c, C3H/Jax, DBA/1, and BDA/2) gave mean erythrocyte values of $8.79–10.52 \times 10^6$ cells per cubic millimeter. The mean cell volumes of the single-type strains ranged from 45.5 to 51.1 $m\mu^3$; those of the diffuse-type strains, from 41.4 to 48.5 $m\mu^3$. Thus neither hemoglobin type is associated closely with any particular level of blood-cell values. Data are also available on the life-span of mice from 11 of these inbred strains (*7*). Mice of all three C57 strains, with single-type hemoglobin, have relatively long life-spans (means for breeding females and males range from 457 to 576 days), but this range is not markedly above that for BALB/c breeders, (mean, 470 days), nor for strain 129 breeders (mean, 556 days), both of which carry diffuse-type hemoglobin. Thus both electrophoretic types of hemoglobin must be regarded as "normal" for mice, with no evidence from blood picture or life-span of selective advantage for either homozygous genotype.

The distribution of hemoglobin type among these inbred strains has an interesting relationship to the history of their development. Figure 1, extended (*8*) and modified from a previous chart prepared by Heston (*9*), summarizes the known history and interrelations of the 20 tested inbred strains, indicating the laboratory stock from which each was derived and the approximate time at which brother-sister inbreeding began. Assuming that no mutations occurred during the course of inbreeding, the alleles now fixed in these strains must have been carried in the original noninbred populations from which they have descended. Of course, these stocks need not have been homozygous for the allele ultimately fixed, nor need the allele even have predominated in the noninbred stock. Five of the single-type inbred strains are descended from a single litter in C. C. Little's colony, derived from the well-known Lathrop-Loeb colony (*9*). Two females, C57 and C58, were mated to their brother, C52. The recently developed WK inbred strain (*10*) is derived from a cross between a C57BL female and a noninbred male heterozygous for *Ww*. Existence of the Hb^1 allele in the littermate females, C57 and C58, or in their brother, C52, would be sufficient to explain the presence of that same gene in these five inbred strains. It is highly improbable that mice of the sixth inbred strain with single-type hemoglobin, SWR, have any ancestry in common with the C57-58 group. The SWR strain was developed by Clara Lynch at the Rockefeller Institute from mice received directly from deCoulon in Switzerland.

There is some common ancestry known for inbred strains characterized by diffuse-type hemoglobin, as is shown in Fig. 1 and in Heston's analysis (*9*). However, the 14 inbred strains with diffuse-type hemoglobin are descended from several different noninbred laboratory mouse colonies (*2a*). The earliest of these noninbred colonies were almost certainly derived independently from the wild or widely separated parts of the Fancy (Little's color stocks and the Ohio dealer's stocks). Furth's A and R stocks may also represent at least one, possibly two, further derivations from the wild. Dunn's color stocks, and the stocks of Waelsch and Holman, may very well have received animals (and consequently hemoglobin-pattern genes) from these early stocks.

The greater frequency of diffuse-type hemoglobin strains suggests wider distribution in nature of the Hb^2 allele. However, it may result from greater propagation in the laboratory, for reasons not related to natural selection, rather than

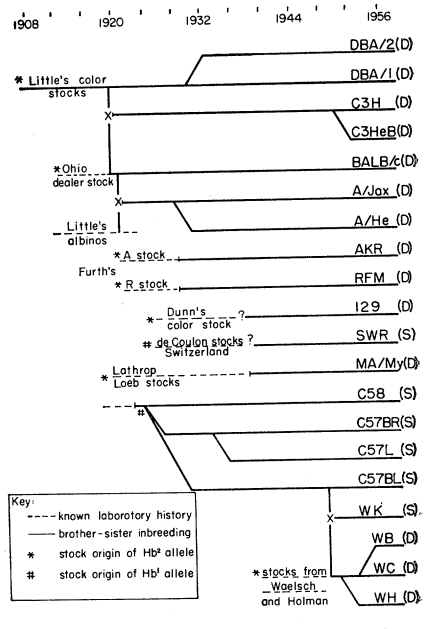

Fig. 1. Known history and interrelations of the 20 inbred strains tested.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

from original greater availability. Mice with either type of hemoglobin appear to be normal and healthy, and both alleles must have been present in wild mice to account for the observed strain distribution.

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References and Notes

1. H. M. Ranney and S. Glücksohn-Waelsch, *Ann. Human Genet.* 19, 260 (1955); S. Glücksohn-Waelsch, H. M. Ranney, B. Sisken, *J. Clin. Invest.* 26, 753 (1957).
2. The symbol for the allele determining single-type hemoglobin is Hb^1; that for the allele determining diffuse-type hemoglobin is Hb^2 (personal communication from S. Glücksohn-Waelsch).
2a. *Note added in proof.* A new study [J. Rosa, G. Schapira, J. C. Dreyfus, J. deGrouchy, G. Mathé, J. Bernard, *Nature* 182, 947 (1958)], by starch-gel electrophoresis, of hemoglobins from five of the same inbred types of mice as those used in the present work, plus one non-inbred type, suggests that there may be at least four electrophoretically distinct types of mouse hemoglobin. If this proves to be true, then there are probably more than the presently postulated two allelic genes determining hemoglobin type, and their distribution would be expected to show less relationship to strain history than would the distribution of only two.
3. P. S. Gerald and L. K. Diamond, *Blood* 13, 61 (1958).
4. H. G. Kunkel, R. Ceppellini, U. Muller-Eberhard, J. Wolf, *J. Clin. Invest.* 36, 1615 (1957).
5. When these determinations involved Jackson Laboratory sublines of strains tested by Ranney and Waelsch, the results agreed completely. We are indebted to Dr. Ranney for preliminary tests of hemoglobin pattern of mice from inbred strains WB, WC, WH, and WK.
6. E. S. Russell, E. F. Neufeld, C. T. Higgins, *Proc. Soc. Exptl. Biol. Med.* 78, 761 (1952).
7. E. S. Russell, "Information on the Inbred Nucleus Strains, Tumors, and Their Uses" (revised), obtainable from Supply Dept., Jackson Laboratory, on request; A. M. Michelson, E. S. Russell, J. Southard, *Am. J. Physiol.*, in press.
8. Committee on Standardized Nomenclature for Inbred Strains of Mice, *Cancer Research* 12, 602 (1952).
9. W. E. Heston, *Jackson Lab. 20th Anniv. Comm.* (1949), p. 9.
10. E. S. Russell, *Genetics* 41, 658 (1956).

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New Type Sedative and Soporific Drug

Abstract. Trimethoxybenzoyl-glycine-diethylamide induced in dogs and cats normal sleep without preceding ataxia. A five- to ten-fold increase of the soporific dose resulted in restlessness and disorientation instead of sleep. In man, oral doses of 500 to 1500 mg caused sedation or drowsiness, or both, in half the cases. No spindling or drug-induced artifacts were found in electroencephalographic recordings.

Drugs commonly used for hypnotic action in the practice of medicine in human beings are ineffective when similarly applied in dogs and cats. These species show ataxia accompanied frequently by frank excitement during induction. Similarly, on spontaneous awakening or active arousal, a similar period of disorganized activity results. In the course of an investigation of new compounds with action on the central nervous system, trimethoxybenzoyl-glycine-diethylamide (Riker 548; proposed generic name, trimeglamide) demonstrated the ability to induce in animals a state of somnolence which could not be distinguished from the physiologic state of sleep. This somnifacient action was neither preceded nor followed by the above-mentioned skeletal muscle involvements. No other drug known to us will produce this phenomenon.

In dogs and cats, the oral soporific dose of trimeglamide was 50 mg/kg; this dose had a latency of 30 to 90 minutes and a duration of 2 to 6 hours. When asleep, the animals could be aroused easily by sound or touch, and they would respond in a normal manner to external stimuli. If left alone, the animals would fall asleep again within a few minutes. There were no indications of skeletal muscle involvement, and no gross abnormalities were detected in neurological examinations. Effects on blood pressure and heart rate were also absent. Rate and depth of respiration remained unchanged.

Larger doses (100 mg/kg) only prolonged the soporific action in cats. In dogs, the soporific effects were also longer lasting and, in addition, some side effects appeared: emesis in about 10 percent of the animals and some muscle twitching and occasional slight ataxia. In addition, a few animals showed definite signs of hyperactivity prior to falling asleep. Raising the dose to 500 mg/kg did not increase the central depressant activity in the dogs nor did it produce sleep, hypnosis, or anesthesia (*1*). Instead, after a brief period of drowsiness or somnolence, a stimulant effect was superimposed upon the soporific action. This state was characterized by restlessness and purposeful locomotion (even though slight ataxia was present in some animals), unusual inquisitiveness, but also some apparent disorientation. Minor obstacles such as a chair leg or a small carton would completely stop the animals, and no attempt would be made to go around or remove the obstacle. Furthermore, the animals frequently would attempt to crawl into almost inaccessible places. As far as could be ascertained, there was no impairment of vision, hearing, taste, or smell. The drug effect gradually disappeared within 2 to 6 hours.

The absence of hypnosis or anesthesia at five to ten times the effective soporific dose in dogs is rather unique and distinguishes trimeglamide from such presently used sedatives as barbiturates, chloral hydrate, methyprylon, ethchlorvynol, and others.

In man, single or repeated oral doses of 500 to 1500 mg caused sedation or drowsiness, or both, without any side effects in about half of over 200 patients thus tested (*2*). Oettinger (*3*) has described the effect of trimeglamide on electroencephalographic recordings. The administration of 2 to 8 mg/kg to 51 children or adults produced in the majority of patients a feeling of relaxation and pleasant tiredness. Electroencephalographic recordings showed neither spindling nor any drug-induced artifacts and no changes in alpha frequency or alpha index.

The lack of hypnosis or anesthesia and of undesirable side effects or artifacts is a distinct advantage for a sleep-inducing drug. In the case of trimeglamide, this advantage is of particular interest since the acute toxicity is very low. Dogs, cats, and mice have tolerated single oral doses of 500, 770, and 2000 mg/kg, respectively. Chronic administration to dogs (35 mg/kg day for 9 months) and rats (100 mg/kg day for 6 months) did not produce signs of drug toxicity during the test or on histopathological examination.

The prolongation of barbiturate-induced sleeping time is considered an index of general central nervous system depression. In mice, sleep induced by pentobarbital sodium (65 mg/kg, intraperitoneal) was increased from 64 ± 11 to 135 ± 21 minutes ($\pm$ standard error) by premedication with 300 mg/kg of trimeglamide given orally 30 minutes before the test. With a subthreshold dose of pentobarbital sodium (30 mg/kg, intraperitoneal) a sleeping time of 13 minutes in 1/20 control mice was increased to 36 ± 9 minutes in 14/40 mice premedicated 30 minutes before the test with an oral dose of 300 mg/kg of the drug. In dogs, with thiopental sodium as the anesthetic, trimeglamide, when given perorally at 20 mg/kg 30 minutes before the test, increased the sleeping time from 20 ± 9 minutes to 46 ± 15 minutes. No apparent effect on respiration was observed. Conversely, premedication with the same dose significantly reduced the amount of thiopental required to abolish the swallowing reflex from 16.8 ± 2.8 mg/kg to 11.3 ± 2.9 mg/kg in a cross-over experiment with eight dogs.

Trimeglamide has anticonvulsant effects in mice against supramaximal electroshock (monophasic rectangular wave, 60 cy/sec, 8.3 msec pulse duration, 0.2 sec shock duration delivered through ocular electrodes). The oral ED_{50} of 510 mg/kg given 30 minutes before the test was about one-sixth of the acute LD_{50}. The drug resembled in this respect other general central nervous system depres-