with no mention of death occurring in either species. Atropine is also commonly employed in man as pretreatment to electroconvulsive therapy. Certain studies(12-15) provide good evidence that when used in doses sufficient to produce peripheral anticholinergic effects, the combination of atropine and electroshock did not result in fatal outcome.

Ability of antispasmodics and ganglionic blocking agents to increase post seizure mortality resembles a similar action of reserpine, epinephrine, chlorpromazine, mescaline, azacyclonol, pipradrol and dibenamine(1,3). Little information bearing on their effectiveness in this respect is available. Such data would be helpful in determining whether post seizure lethal action of this diverse group of substances is nonspecific or whether a single property or effect underlies their action.

Summary. Ability of several antispasmodic and ganglionic blocking substances to produce immediate death following maximal electroshock convulsions in mice has been demonstrated. With most agents studied (atropine, atropine methyl nitrate, benactyzine, mepiperphenidol, methantheline, propantheline, scopolamine, scopolamine methiodide, mecamylamine, chlorisondamine, pentolinium and hexamethonium), the post seizure lethal effect was produced in doses approximating that required to produce a peripheral anticholinergic effect in the same species (pupil dilatation). Neither atropine nor mecamylamine altered the electroconvulsive threshold.

Respiratory failure appeared to be the cause of drug-induced post seizure deaths, but the mechanism of action was not revealed.

1. Jenney, E. H., *Fed. Proc.*, 1954, v13, 370.
2. Berger, F. M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 563.
3. Toman, J. E. P., Everett, G. M., from H. Pennes "Psychopharmacology", 1958, Hoeber-Harper, N. Y., p247.
4. Swinyard, E. A., Brown, W. C., Goodman, L. S., *J. Pharmacol. & Exp. Therap.*, 1952, v106, 319.
5. Litchfield, J. T., Jr., Wilcoxon, F., *ibid.*, 1949, v96, 99.
6. Pulewka, P., *Arch. exper. Path. u. Pharmakol.*, 1952, v168, 307.
7. Stone, C. A., Meckelnburg, K. L., Torchiana, M. L., *Arch int. pharmacodyn.*, 1958, v117, 419.
8. Weil, C. S., *Biometrics*, 1952, v8, 249.
9. Torchiana, M. L., Stone, C. A., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 290.
10. Wolf, J., *Arch. exper. Path. u. Pharmakol.*, 1951, v214, 14.
11. Wienke, H., *ibid.*, 1953, v217, 312.
12. Altschule, M. D., Tillotson, K. J., *Arch. Neurol. and Psychiat.*, 1948, v60, 392.
13. Hejtmancik, M. R., Barklead, A. J., Herrmann, G. R., *Amer. Heart J.*, 1949, v37, 790.
14. Impastato, D. J., *Dis. Nerv. System.*, 1957, v18, 34.
15. Ulett, G. A., Johnson, M. W., *EEG J.*, 1957, v9, 217.

Received May 20, 1959. P.S.E.B.M., 1959, v101.

Solubility of Hemoglobin as Red Cell Marker in Irradiated Mouse Chimeras. (25084)

RAYMOND A. POPP AND G. E. COSGROVE
(Introduced by A. C. Upton)
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.*

Transplantation and growth of donor-type progenitors of mouse erythrocytes in lethally irradiated mice may be demonstrated by serological markers(1) when donor and recipient red cells are of different *H-2* genotypes, and by agar(2) and starch gel electrophoresis(3) when their hemoglobins give different patterns on electrophoresis. This report describes differences in solubility of various mouse hemoglobins that can be used to identify erythrocytes of genetically different types probably not distinguishable from one another serologically or electrophoretically.

Materials and methods. Blood from de-

* Operated by Union Carbide Corp. for U. S. Atomic Energy Comm.

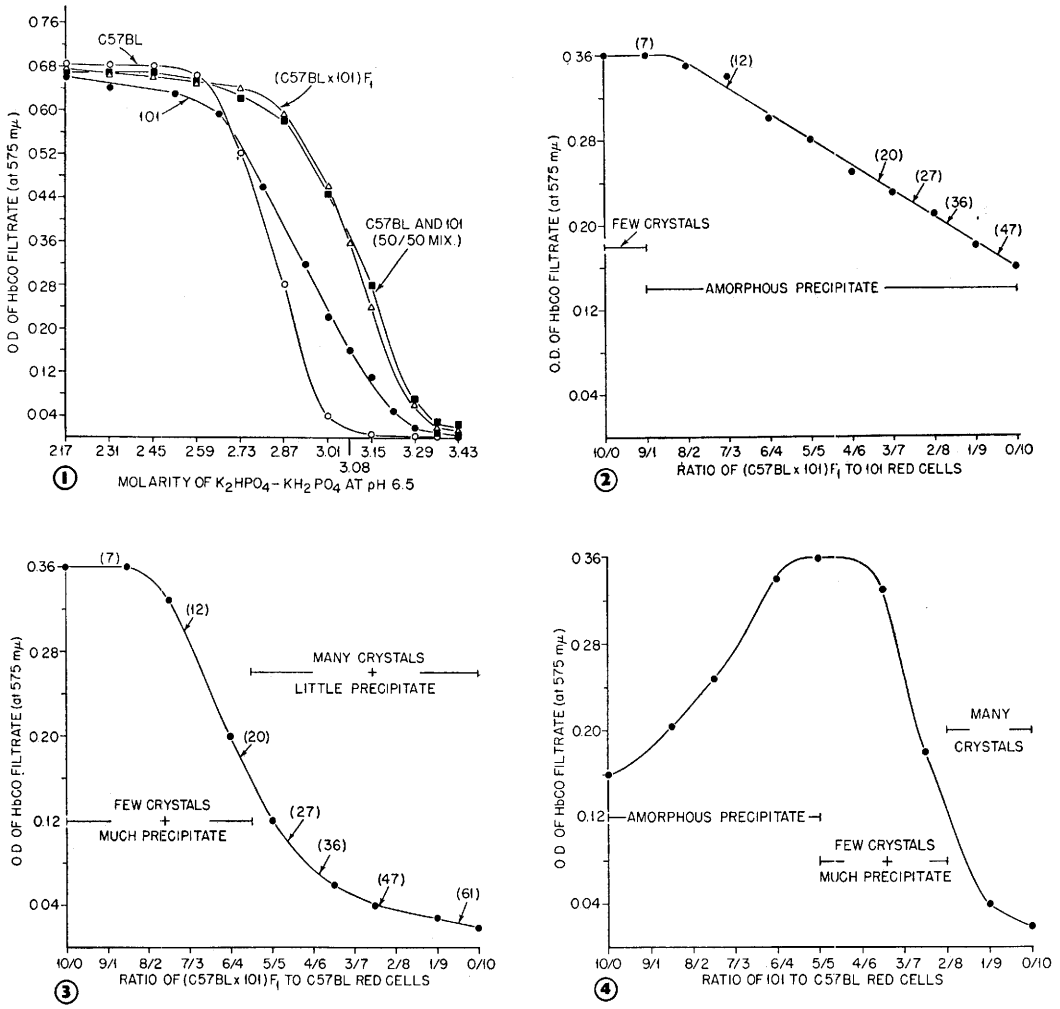

FIG. 1. Salting out curves for genetically different mouse hemoglobins. Preparations contained 0.1 ml of HbCO in total volume of 5 ml, distilled water added as necessary to change molarity of phosphate buffer. Readings were made at 21 hr (readings made prior to 18 hr were slightly higher and those after 24 hr slightly lower owing to incomplete salting out and gradual denaturation).

FIG. 2. HbCO solubility in irradiated (C57BL × 101)F_1 mice receiving transplants of 101 bone marrow. Arrows show avg O.D. found and numbers in parentheses show days after irradiation on which chimeras were tested. Data were obtained from 6 of 10 surviving lethally irradiated recipients receiving 15 × 10^6 donor bone marrow cells.

FIG. 3. HbCO solubility in irradiated (C57BL × 101)F_1 mice receiving transplants of C57BL bone marrow. For complete explanation of notations see footnote in Fig. 2. Results from 10 lethally irradiated recipients receiving 15 × 10^6 donor bone marrow cells, only 6 of which survived through day 36.

FIG. 4. Solubility of mixtures of 101 and C57BL HbCO. Shape of this curve clearly indicates that equilibrium solubility is not reached under conditions used; however, these conditions are preferred because of rapid denaturation of labile mouse hemoglobin.

capitated mice was suspended in about 200 vol of cold physiological saline. Red cells were concentrated by centrifugation, the saline was siphoned off, and the cells were washed 3 more times, each time cells were resuspended in 10 vol of saline. Packed erythrocytes were then lysed in an equal volume of distilled water, and red cell membranes were removed by centrifugation. The supernatant oxyhemoglobin (HbO_2) was converted to carbon monoxyhemoglobin (HbCO) by bubbling CO through the solution until the deep red color of HbO_2

TABLE I. Comparison of Hemoglobin of Various Mouse Strains as Regards Genotype, Electrophoretic Pattern, Solubility, and Type of Precipitate Formed.

Strain*	Genotype†	Electrophoretic pattern‡	Solubility (O.D.)§	Precipitate‖
C57BL C57L C57BR pc^{ch} 11G/Rl	Hb^1 Hb^1	Single	.01–.02	Crystalline
101 AK C3H DBA/2 RF (101 × C3H)F_1	Hb^2 Hb^2	Diffuse	.14–.16	Amorphous
(C57BL × 101)F_1 (C57BL × DBA/2)F_1 (C57L × A)F_1	Hb^1 Hb^2	"	.33–.36	Mixed¶

* Minimum of 6 mice checked for each group.
† After Gluecksohn-Waelsch (see Russell, E. S., Gerald, P. S., *Science*, 1958, v128, 1569).
‡ Nomenclature of Gluecksohn-Waelsch *et al.*(5).
§ Avg O.D. of HbCO at 575 mμ (see text).
‖ See Fig. 5.
¶ Crystals in (C57BL × 101)F_1 more numerous at higher concentrations of phosphate buffer.

became the brilliant crimson color of HbCO. Derrien's technics(4) were used to determine the solubility of HbCO in K_2HPO_4-KH_2PO_4 buffer at pH 6.5. Salting out preparations stood for 18-24 hours at room temperature before they were filtered through Whatman No. 1 filter paper. Optical density (O.D.) of the filtrates was read at 575 mμ in a Coleman Jr. Spectrophotometer, Model 6A. Precipitate formations were examined under a microscope at 430 X.

Results. Fig. 1 shows the salting-out curves for HbCO prepared from erythrocytes of C57BL, 101, and (C57BL X 101)F_1 mice. Hemoglobins of various other strains exhibited a similar relation between solubility and electrophoretic mobility (Table I). Comparable results were obtained when a few drops of whole blood from tail vein were placed in distilled water and the solution was bubbled with CO before phosphate buffer was added. Mixtures of similar types of hemoglobins [*e.g.*, C3H + 101, C57L + C57BL, or (C57BL X 101)F_1 + (C57BL X DBA/2)F_1] had solubilities characteristic of individual types being mixed, but mixtures of different HbCO types gave O.D. readings intermediate between those of original HbCO samples, increased, or decreased, depending on relative percentages of the two HbCO types in the mixture (Fig. 2, 3, and 4). On salting out, HbCO of the homozygous-diffuse type formed an amorphous precipitate, that of the single-type formed large hexagonal crystals, and that of the heterozygous-diffuse type formed a mixture of amorphous precipitate and crystals (Fig. 5). Crystals also developed in mixtures of single and homozygous-diffuse HbCO when more than 50% of the HbCO was of single-type. Optical densities of known mixtures of HbCO filtrates were measured, and precipitates were examined to determine how solubility and precipitate formation would correlate with changes in erythrocyte population during establishment of red cell chimerism. The results obtained from lethally irradiated (C57BL X 101)F_1 mice that received injections of either C57BL or 101 bone marrow cells are compared in Figs. 2 and 3 with values obtained from known HbCO mixtures. Comparative analyses of erythrocytes of chimeras with both starch gel electrophoresis and hemoglobin solubility indicated that the results of the 2 technics agreed with one another (Table II).

Discussion. The O.D. of the HbCO filtrate is an expression of solubility of HbCO at a specific pH and salt concentration. Since K_2HPO_4–KH_2PO_4 at pH 6.5 has a large buffering capacity, pH of the solution remains essentially constant as HbCO is salted out. Small differences in salt concentration, how-

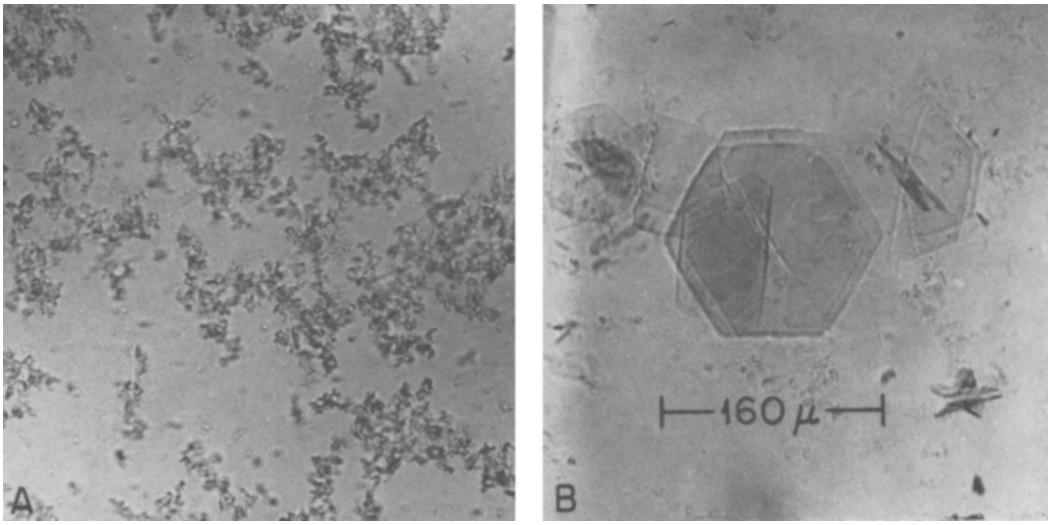

FIG. 5. A. Amorphous precipitate obtained with 101 HbCO; B. crystals formed with C57BL HbCO. Crystals were formed in 2.87 M phosphate, at which molarity size and definition seems to be maximal.

ever, cause significant changes in HbCO solubility (Fig. 1). The quantity of HbCO used should be sufficient to give final O.D. readings of at least 0.50 in low salt concentrations. Initial O.D. values of our preparations were 0.90-1.00 and final readings were about 0.65-0.70 after the preparations had stood for 18-24 hours at room temperature in 1.75 M phosphate buffer. Although under these conditions HbCO apparently did not salt out, some nonspecific denaturation of labile mouse hemoglobin nevertheless occurred. HbCO was used because it was more stable than HbO_2. If large concentrations of HbCO were used, a longer time was required to salt out the excess HbCO. Filtrates of supersaturated HbCO solutions became turbid upon standing, owing to further precipitation of HbCO. Results were more reproducible when initial concentrations of HbCO were similar, perhaps because of more-uniform gradual nonspecific denaturation as well as salting out of mouse HbCO. Quantitative determinations of the mixtures of red cells in chimeras were made with about 0.1 ml of whole blood from the severed tail vein. Blood from anemic animals frequently gave readings lower than expected since initial concentration of HbCO was less than optimal.

Possible uses of hemoglobin as a red cell marker, particularly where *H-2* markers cannot be used, have been discussed(3). The use of hemoglobin solubility is even more adaptable than electrophoresis for assay of erythrocytes in irradiated mouse chimeras since all 3 hemoglobin genotypes can be identified by their solubility differences (Table II). For example, although presence of strain 101 red cells in group B and absence of such cells in group C cannot be determined by electrophoresis, they can be readily determined by HbCO solubility. The percentage of cells of one type in mixed populations of erythrocytes can also be estimated as well by solubility as by our previously reported electrophoretic technic(3). A major drawback in the quantitation, however, is that similar optical densities from 0.16-0.36 are obtained for 2 very different populations of red cells when mice with single and homozygous-diffuse hemoglobin types are used as donors and recipients of bone marrow (Fig. 4). In this situation, the correct choice can be ascertained only by examining the precipitate or by noting the type of red cells in the populatiin that is increasing on successive weekly readings.

The genetic basis for occurrence of more than one hemoglobin in the mouse seems to be similar to that in man(5). The similarity of salting-out curves for heterozygous-diffuse

TABLE II. Identification of Hemoglobin in Irradiated Mouse Chimeras.

Group	No. of recipient mice*	Dose (r)	Donor nucleated cells ($\times 10^6$) Bone marrow† C57BL	Bone marrow† 101	Spleen‡ C57BL	Spleen‡ 101	Post-treatment (mo)	Chimera hemoglobin — Electrophoretic mobility§	Chimera hemoglobin — Optical density‖
A	2	900	15				3	Single	.039 ± .011
	5	500			120		3	"	
B	16	900		15			2–3	Diffuse	.18 ± .008
	1	600				120	3	"	
C	13	400–600			120		2–4	"	.40 ± .012
	10	"				30	2–3	"	
	3	600				12	2	"	

* (C57BL × 101)F_1; 12-16 wk old. † Inj. intrav. ‡ Inj. intraper. § As determined by starch gel electrophoresis, nomenclature of Huecksohn-Waelsch *et al.*(5). ‖ Avg O.D. ± S.E. of mouse HbCO filtrate (see text).

HbCO and for equal mixtures of single with homozygous-diffuse HbCO (Fig. 1) suggests that each hemoglobin gene expresses itself in hybrids by controlling the synthesis of its own hemoglobin type. The development of crystals of single-type hemoglobin in mixtures of HbCO of different genotypes (see Figs. 2, 3, 4) is consistent with this hypothesis. In this respect the action of the hemoglobin genes of the mouse is similar to that of genes governing hemoglobin synthesis in man(6,7).

Summary. Genetically different mouse hemoglobins differ in solubility and crystal formation. Differences in hemoglobin solubility and crystal formation can therefore be used to identify homologous erythrocytes in irradiated mouse chimeras if the hemoglobin types of donor and recipient mice are distinguishable by these properties to begin with.

We wish to acknowledge technical assistance of D. E. Reynolds.

1. Owen, R. D., *Radiation Res.*, 1958, v9, 164.
2. Welling, W., van Bekkum, D. W., *Nature*, 1958, v182, 946.
3. Popp, R. A., Cosgrove, G. E., Owen, R. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 692.
4. Derrien, Y., *Biochim. Biophys. Acta*, 1952, v8, 631.
5. Huecksohn-Waelsch, S., Ranney, H. M., Sisken, B. F., *J. Clin. Invest.*, 1957, v36, 753.
6. Neel, J. V., *Science*, 1949, v110, 64.
7. Itano, H. A., in Anfinsen, C. B., Jr. *et al.*, Editors, *Advances in Protein Chemistry*, Vol. XII, Academic Press, N. Y., 1957, p215.

Received May 20, 1959. P.S.E.B.M., 1959, v101.

Effect of Dietary Zinc Deficiency of Chicken Hepatic Cells. (25085)

W. G. HUBER,* G. L. WAXLER, M. W. MOELLER AND H. M. SCOTT
(Introduced by C. A. Brandly)
Ill. Agric. Exp. Station, University of Illinois, Urbana, and College of Veterinary Medicine, Mich. State University, East Lansing

O'Dell and Savage(1,2) reported that growth of chicks was stimulated when as little as 6.6 ppm of zinc was added to a purified diet of glucose-isolated soybean protein (Drackett Assay Protein C-1) type even though the basal contained about 50 ppm of Zn. Further reports concerned with zinc requirements of chicks and poults fed highly refined diets have been reported by Norris *et al.*(3), Morrison and Sarett(4), Pensack *et al.*(5), Norris and Zeigler(6), Roberson and Schaible(7) and Supplee *et al.*(8). This communication reports histopathological findings observed in chicks fed a diet deficient in zinc 14 days following hatching.

Materials and methods. Crossbred male

* Present address: Chas. Pfizer & Co., Terre Haute, Ind.