Table 1. Compensation and incubation effect with physiological and synthetic sphingosine. The composition of the reaction mixtures was as follows: lipid activator, 30 mm^3 (6 mg/1 ml of 0.85-percent NaCl); plasma, 150 mm^3; natural sphingosine, 30 or 60 mm^3 (5 mg/1 ml of H_2O); synthetic sphingosine, 30 or 60 mm^3 (1, 25 mg/1 ml of H_2O); all volumes were made up to 270 mm^3 with either H_2O or NaCl (0.85 percent) as needed to obtain equivalent amounts of each in the final reaction mixture. Chicken plasma obtained from the carotid (method of Carrel) was used throughout. Clotting time was assayed by the method of Hecht (*7*).

Expt. No.	Composition of reaction mixture	Clotting time
1	Physiological NaCl solution + plasma	30 min, 0 sec
2	Lipid activator (30 mm^3) + plasma	4 min, 50 sec
3a	Physiological sphingosine (30 mm^3) + plasma	> 40 hr
	Physiological sphingosine (60 mm^3) + plasma	> 40 hr
3b	Synthetic sphingosine (30 mm^3) + plasma	> 40 hr
	Synthetic sphingosine (60 mm^3) + plasma	> 40 hr
4a	Physiological sphingosine (30 mm^3) + plasma + lipid activator directly	5 min, 0 sec
	Physiological sphingosine (60 mm^3) + plasma + lipid activator directly	15 min, 15 sec
4b	Synthetic sphingosine (30 mm^3) + plasma + lipid activator directly	4 min, 20 sec
	Synthetic sphingosine (60 mm^3) + plasma + lipid activator directly	6 min, 10 sec
5a	Physiological sphingosine (30 mm^3) + plasma + lipid activator added after incubation for 1 hr at 39°C	1 min, 8 sec
	Physiological sphingosine (60 mm^3) + plasma + lipid activator added after incubation for 1 hr at 39°C	1 min, 25 sec
5b	Synthetic sphingosine (30 mm^3) + plasma + lipid activator added after incubation for 1 hr at 39°C	43 sec
	Synthetic sphingosine (60 mm^3) + plasma + lipid activator added after incubation for 1 hr at 39°C	58 sec

gosine compensation." If the chicken plasma is first incubated for 1 hour at 39°C with sphingosine, the clotting times with the lipid activator are one-third to one-sixth of those obtained with the optimal concentration of the lipid activator alone. This is called "incubation effect." This effect is not seen if the plasma is added to the incubated mixture of sphingosine and the lipid activator (*2*). Therefore we assume the presence of an unidentified component in the plasma which reacts with the inhibitor to produce the incubation effect.

Fortunately, sphingosine and certain derivatives have been synthesized (*6*). The structure of sphingosine is as follows: $CH_3—(CH_2)_{12}—CH=CH—CH(OH)—CH(NH_2)—CH_2OH$. DL-sphingosine has been studied in connection with chicken plasma, and all the reactions that occur with the use of concentrates from biological sources are seen with the synthetic preparation. However, smaller quantities suffice.

The intensity of the reactions with sphingosine depends largely on its concentration, on the concentration of the lipid activator, and on individual properties of the plasma. Synthetic *erythro-* and *threo*-dihydrosphingosine are weak inhibitors and give only a slight sphingosine compensation. Both substances may prolong slightly the clotting time of chicken plasma. They raise the activity of the lipid activator to a slight degree and, when incubated with plasma, produce very short clotting times. N-benzoyldihydrosphingosine is completely inactive, as is also the tri-acetyl derivative of sphingosine.

Our experiments give the information that the presence of the double bond, as well as the presence of the free functional hydroxyl and the amino groups, is essential for the clot-delaying activity.

We were also able to synthesize the two isomers of threoninol, which are the lowest homologs of dihydrosphingosine and which have the following structure; $CH_3—CHOH—CHNH_2—CH_2OH$. The free threoninols are indifferent in every respect. Their oxalates have a powerful inhibitory influence on the clotting of chicken plasma. This can be abolished by the lipid activator, but not after preliminary incubation of the oxalates with the plasma. Table 1 summarizes the clotting times of chicken plasma that has been treated with natural and synthetic sphingosine only.

E. HECHT

Medical Department, University Hospital, Utrecht, Netherlands

D. SHAPIRO

Daniel Sieff Research Institute, Weizmann Institute of Science, Rehovoth, Israel

References

1. E. Hecht, *Nature* **167**, 279, 633 (1951).
2. ——, *Acta Haematol.* **9**, 237 (1953).
3. A. Fischer and E. Hecht, *Biochem. Z.* **269**, 115 (1934).
4. E. Hecht, *Acta Physiol. et Pharmacol. Neerl.* **2**, 134 (1951).
5. L. M. Tocantins and R. T. Carroll, *Blood Clotting and Allied Problems* (Josiah Macy, Jr., Foundation, New York, 1949), pp. 11–28.
6. D. Shapiro and K. Segal, *J. Am. Chem. Soc.* **76**, 5894 (1954); D. Shapiro, H. M. Flowers, E. Hecht, *J. Org. Chem.*, in press.
7. E. Hecht, *Acta Med. Scand.* **102**, 79 (1939).

14 January 1957

Reaction of 8-Quinolinol and 2-Methyl-8-Quinolinol with Rare-Earth Elements

In an attempt to improve the selectivity of 8-quinolinol as an analytic precipitant, various substituted compounds have been proposed. Merritt and Walker (*1*) found that 2-methyl-8-quinolinol did not precipitate aluminum ion; thus it offered some selectivity over 8-quinolinol. Irving, Butler, and Ring (*2*) showed that this increased selectivity was the result of the steric hindrance effect of the methyl group at the 2-position. This effect would also slightly alter the sensitivity of the reaction of this reagent with other metal ions.

From the results of this previous work, one would predict that 2-methyl-8-quinolinol would perhaps be less sensitive in the detection of the trivalent rare earth metal ions than 8-quinolinol. Under the same conditions of hydrogen-ion and chelating-agent concentrations, this has been found to be true.

To determine the sensitivity of the chelating agents, a modification of the Irving, Butler, and Ring (*2*) method was used. An NH_4Cl-NH_4OH buffer was used because, with the buffers recommended in the original method, only 8-quinolinol gave precipitates with the metal ions (*p*H 8.4). The new buffer consisted of 100 g of NH_4Cl, 60 ml of concentrated NH_4OH, and 440 ml of water. The *p*H of a solution containing 2 ml of this buffer in a total volume of 6.2 ml was 9.5. Concentrations of the rare-earth chloride solutions were 0.01 and 0.001*M*. The chelating agents were 0.1*M* in 95-percent ethanol. Test solutions were heated 10 minutes at 70°C, then after they had cooled to room temperature, they were observed visually for signs of a precipitate.

The results of the sensitivity tests are given in Table 1. The concentrations of metal ions are expressed in micrograms of M^{+3} per milliliter. It can readily be seen that under the same conditions, the sensitivity of 2-methyl-8-quinolinol in precipitating the rare-earth metal ions is much less than that of 8-quinolinol. Quantitatively, the former chelating agent averaged about one-eighteenth as sensitive as the latter.

Table 1. Results of sensitivity tests.

Metal ion	8-Quinolinol	2-Methyl-8-quinolinol
Lanthanum	0.91	36
Cerium	0.93	18
Praseodymium	3.7	37
Neodymium	3.7	37
Samarium	1.9	39
Gadolinium	1.0	6.0
Yttrium	0.57	23
Scandium	1.2	2.9

The theoretical basis for sensitivity tests has been discussed by Irving and Rossotti (*3*). Although quite complex, the sensitivity of a precipitation reaction depends on the intrinsic solubility of the chelate and also the stability of the metal chelate. The intrinsic solubility of the chelate, in turn, is subject to the structural factors of the chelating agent. Since the introduction of a methyl group on the 8-quinolinol molecule should not alter the solubility of the metal chelates appreciably, the main factor here must be the stability of the chelate. Further work is needed to evaluate the stability constants of these chelates.

WESLEY W. WENDLANDT
Department of Chemistry and Chemical Engineering, Texas Technological College, Lubbock

References

1. L. L. Merritt and J. K. Walker, *Ind. Eng. Chem. Anal. Ed.* **16**, 387 (1944).
2. H. Irving, E. J. Butler, M. F. Ring, *J. Chem. Soc.* **1949**, 1489 (1949).
3. H. Irving and H. S. Rossotti, *Analyst* 80, 245 (1955).

4 March 1957

First Example of Genotype $r^y r^y$—a Family Study

In 1954 blood of a D-negative patient (F.S.) was submitted with a history of hemolytic disease of the newborn. The patient—the propositus—had two normal children followed by two infants with severe hemolytic disease. The infant born in 1952 died from kernicterus at 72 hours, while the infant born in 1954 recovered following numerous small transfusions. The serum of the propositus, when tested during the fourth pregnancy, contained anti-D (anti-Rh_0) to a titer of 1/128.

The blood of the propositus, II-2 in Fig. 1, failed to react with anti-D serums, but it reacted with anti-C and anti-E serums. The absence of reactions with several different anti-c and anti-e serums suggested that the gentoype of the propositus was $r^y r^y$ (*dCE/dCE*). The negative reactions for D^u and the production of anti-D further confirmed that the patient was D-negative. Because genotype $r^y r^y$ (*dCE/dCE*) had never been observed previously (*1*) studies were carried out with all available members of three generations shown in Fig. 1.

Only nine of the 12 members were available for testing, and a summary of the findings is presented in Table 1. The reactions were confirmed in tests with numerous examples of anti-D, anti-E, and anti-c and fewer samples of anti-C and anti-e. For the blood of the propositus, II-2, the following reagents were employed: 10 anti-D saline (complete) agglutinins, 16 anti-D albumin (incomplete) agglutinins, 11 anti-C, 12 anti-E, 8 anti-c, and 6 anti-e serums. Each of the 16 albumin (incomplete) agglutinins failed to react with the blood of the propositus both in the direct tests and in the antiglobulin test. These negative reactions further established the absence of the weakly reacting D^u in the blood of the propositus. Similarly, the absence of factors c and e was definitely proved by negative direct and indirect antiglobulin reactions.

From the data in Table 1, shown also in Fig. 1, it is noted that, excluding the husband of the propositus, there are seven members whose blood contains the very rare chromosome r^y (*dCE*)—the propositus who is $r^y r^y$ (*dCE/dCE*) and six who are heterozygous for r^y (*dCE*), associated with chromosome *r* once, with

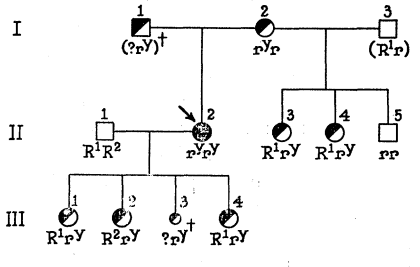

Fig. 1. Family tree with seven members containing the rare chromosome r^y (*dCE*) and the first example of genotype $r^y r^y$ (*dCE/dCE*).

Table 1. Summary of reactions with five antiserums. Siblings II-3, II-4, and II-5 were products of the second marriage. Their father was not available for testing.

Member	Anti- D	C	E	c	e	Genotype	
I-2 Mother	o	+	+	+	+	*dCE/dce*	$r^y r$
II-1 Husband of propositus	+	+	+	+	+	*DCe/DcE*	R^1R^2
II-2 Propositus	o	+	+	o	o	*dCE/dCE*	$r^y r^y$
III-1	+	+	+	o	+	*DCe/dCE*	$R^1 r^y$
III-2	+	+	+	+	o	*DcE/dCE*	$R^2 r^y$
III-4	+	+	+	o	+	*DCe/dCE*	$R^1 r^y$
II-3 Sibling	+	+	+	o	+	*DCe/dCE*	$R^1 r^y$
II-4 Sibling	+	+	+	o	+	*DCe/dCE*	$R^1 r^y$
II-5 Sibling	o	o	o	+	+	*dce/dce*	*rr*

R^1 four times, and once with R^2. In addition, r^y (*dCE*) must have been present in the blood of I-1, the deceased father of the propositus, and also in the fatally affected infant III-3.

This family tree, excluding the random I-3 and II-1 (whose genotype can be constructed) consists of 10 individuals, and among them there are 10 r^y (*dCE*) chromosomes, the absence in II-5 being neutralized by its presence in double dose in the propositus II-2. As could have been anticipated from the rarity of the chromosome involved, the parents of the propositus, I-1 and I-2, were consanguineous, each contributing one r^y (*dCE*) chromosome to the propositus.

This unique family provided an opportunity to study the suppression of D by its partner chromosome containing factor C, first described by Ceppellini, Dunn, and Turri (*2*). This suppression is exerted not only by chromosome r' (*dCe*) but also by chromosome r^y (*dCE*). A new variety of suppression of factor c in certain combinations was also observed (*3*).

ROBERT MCGEE
PHILIP LEVINE
MARIO CELANO
Ortho Research Foundation, Raritan, New Jersey

References and Notes

1. R. Race and R. Sanger [*Blood Groups in Man* (Thomas, Springfield, Ill., 1954)] estimate that chromosome r^y (*dCE*) should have a frequency of 1 in 48 million. Thus, one homozygote can be expected in testing about 400 million individuals in Western Europe. A. S. Wiener [*Am. J. Human Genet.* 1, 127 (1949)] calculates that the homozygote has an incidence of 1 in 100 million.
2. R. Ceppellini, L. C. Dunn, M. Turri, *Proc. Natl. Acad. Sci. U.S.* 41, 283 (1955).
3. A discussion of these findings is in preparation.

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