A New Blood Factor, s, Allelic to S. (19025)

PHILIP LEVINE, A. B. KUHMICHEL, M. WIGOD, AND E. KOCH.
From the Ortho Research Foundation, Raritan, N. J.

On testing the serum of a mother who delivered an infant suffering with hemolytic disease, atypical reactions were observed which did not conform in specificity with any of the known antibodies. The mother, Mrs. Audrey Guth, para 2, gravida 3, had a normal full-term infant, a subsequent abortion at 2 months of pregnancy and on October 27, 1950 a delivery of a severely affected infant, who recovered following 2 transfusions of Rh negative blood.* Preliminary tests showed the father and the affected infant to be in group O, Rh negative (cde/cde), and the mother Rh positive, type Rh_2 (cDE/cde). Titration of the mother's serum on the husband's cells showed the presence of very weak saline agglutinins and incomplete antibodies, demonstrable at about 1:8 with the indirect Coombs test and in tests with trypsinized cells.

Additional studies could not be carried out until April 1951, when the child was 4 months old and free from symptoms. More complete determination of the antigenic components of the red cells of the 4 members of the family are given in Table I.

The mating was incompatible for N, Kidd(1), and more significantly for the factor demonstrable with the antibody in the mother's serum. The antibody 4 months post partum gave weak or negative reactions with saline suspended cells, and its titer in the indirect Coombs was about 1:4. Parallel tests with a panel of selected red cells failed to reveal any parallelism in the reactions of anti-N, anti-Kidd, and the antibody in the Guth serum which gave about 88% positive reactions. These preliminary studies indicated the existence of a new blood factor which induced transplacental isoimmunization and hemolytic disease.

A clue to the specificity of the antibody was revealed on testing the antigenic constitution of all bloods lacking this new blood factor. Remarkably enough, all these bloods gave strong and distinct reactions with anti-S.† In short, on testing numerous bloods with the Guth serum and anti-S, only 3 types of reactions were observed as in the case of M-N, C-c, E-e, and K-k systems. A type of blood lacking both S and the new factor was never observed in tests of more than 800 bloods. On the basis of preliminary findings, the factor was called "s" and its antibody "anti-s."

Since the titer of anti-s 4 months postpartum was low, 3 intravenous injections of 1-2 cc of the husband's blood were administered, as a result of which the titer was increased to 1:32-1:64 in the indirect Coombs test. The titer with saline suspended cells, which was 1:32-1:64 after 2 injections dropped after the third injection to a value of 1:4-1:8 on homozygous bloods ss.

With this more potent anti-s at hand, tests were carried out in parallel with it and anti-S on 353 random white and 145 random colored

* For the specimens in this case the authors are indebted to Drs. W. R. Platt, pathologist, and R. A. Warwick, pediatrician, at the West Jersey Hosp., Camden, N. J.

† The S factor was discovered in 1947 by the Australian workers Walsh and Montgomery with the aid of an antibody present along with anti-Rh in the serum of a mother of an erythroblastic infant(2). Its relationship to the MN system was described by Sanger and Race in a study of the Australian serum(3). Several examples of anti-S were described either as naturally occurring antibodies or as immune antibodies following a transfusion. Anti-S occurring by itself as the cause of hemolytic disease has as yet not been observed (4).

1. Allen, F. H., Diamond, L. K., and Niedziela, B., *Nature,* 1951, v167, 482.

2. Walsh, R. J., and Montgomery, C., *Nature,* 1947, v160, 504.

3. Sanger, R., and Race, R. R., *Nature,* 1947, v160, 505.

4. Levine, P., *Trans. New York Acad. Sciences,* 1951, v13, 205.

TABLE I. Bloods of the Guth Family Studied with 15 Different Sera.

			D	C	E	c	e	K	k	Duffy	Kidd	S	Mrs. Guth
Father	O	N	0	0	0	+	+	0	+	0	+	0	+
Mother	O	M	+	0	+	+	+	0	+	0	0	+	0
1st child	O	MN	+	0	+	+	+	0	+	0	+	+	+
Affected infant	O	MN	0	0	0	+	+	0	+	0	*	+	+

* Not tested.

TABLE II.

Anti-S	Anti-s	White (%)	Colored (%)	Genotype
+	0	12.18	2.76	*SS*
+	+	46.46	28.96	*Ss*
0	+	41.36	68.28	*ss*
No. studied		(353)	(145)	

individuals. The incidence of the three types of bloods in the 2 groups is given in Table II.

The genetic relationship is further revealed by the gene frequencies, the calculation of which is given below:

Frequency of gene			White	Colored
S	=	$\sqrt{SS}$	.349	.166
s	=	$\sqrt{ss}$	.643	.827
			.992	.993

The values for anti-S in the colored population, *i.e.* 31.72% (SS plus Ss), are in good agreement with that observed by Miller, Rosenfield, and Vogel(5) in a sample of 580 colored individuals *i.e.* 29.5%.

The values of the gene frequencies for the white population in the United States do not differ much from those obtained by Race and his co-workers(6) in their studies on the distribution of only one of these factors, S, in an English population.

Frequency of gene	Race, *et al.*	Present study
S	.337	.349
s	.663	.643

The genetic relationship is further revealed in the so-called double-dose effect of antigenic factors shown by the direct reaction of anti-s both after the second injection and after the third injection when the direct saline titer was considerably lower. With the latter specimen, almost all homozygous bloods were selected by direct reactions with saline suspended cells while distinct reactions for heterozygous bloods could be elicited only after application of the indirect Coombs test.‡

Prior to the discovery of the s factor, it was not clear whether the genetic relationship of the S factor to the MN systems is that of multiple alleles at one locus or of close linkage. The existence of the s factor would seem to indicate linkage as in the case of the CDE/cde factors rather than multiple alleles. Additional evidence to support this view will be published by Sanger and Race(7) to whom a supply of anti-s was made available for testing some of the 123 families included in their genetic study of the factors M, N, and S.

It is hoped that sufficient supplies of potent samples of both anti-S and anti-s will soon become available for comprehensive family and racial studies. As predicted by Fisher (8), the MNSs system is more efficient than either the ABO or Rh-Hr systems for the differentiation of human blood and for rendering decisions of non-paternity. Incidentally, it may be mentioned that the notation of the genes *M* and *N* will eventually have to be altered to make them conform with genetic usage.

Summary. A new blood factor, s, is de-

5. Miller, E. B., Rosenfield, R. E., and Vogel, P., *Am. J. Phys. Anthrop.*, in press.

6. Race, R. R., Sanger, R., Lawler, S. D., and Bertinshaw, D., *Heredity*, 1949, v3 part 2, 205.

‡ The double dose effect for direct selection of individuals homozygous for S could not be demonstrated with anti-S used in these studies. For a supply of this serum, the authors are indebted to both Drs. Vogel and Rosenfield and to Dr. Mourant.

7. Sanger, R., and Race, R. R., in preparation.

8. Fisher, R. A., cited by Race *et al.*(6).

scribed with the aid of an antibody produced by transplacental isoimmunization. Evidence is presented to show its genetic relationship to the S factor, which is probably linked to the M and N system.

Received August 23, 1951. P.S.E.B.M., 1951, v78.

Quantitative Biologic Activity of $\Delta^{4,6}$ dehydrocortisone as Compared to Cortisone.* (19026)

DWIGHT J. INGLE, ERVING H. MORLEY, AND JAMES E. NEZAMIS.
From the Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

The compound $\Delta^{4,6}$dehydrocortisone acetate was first recognized as a byproduct of the partial synthesis of cortisone acetate(1). It is commonly designated as the "diene" of cortisone. The diene is important from the standpoint of establishing correlations between the chemical structure of steroids and their biologic and therapeutic effects. In a preliminary report, Kendall(1) noted that the diene did not suppress the symptoms of rheumatoid arthritis, although its glycogenic potency seemed to equal that of cortisone. Some apparent differences in the physiologic properties of the two compounds were reported by Higgins, Woods, and Kendall(2).

In the present study we have compared the potency of cortisone acetate and the diene acetate in the muscle-work test of Ingle(3) and in the glycogen deposition test. According to these criteria the quantitative potency of the diene acetate is probably no greater than 50% of the potency of cortisone acetate.

Methods. Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets until they reached a weight of 200 ± 2 g. The procedures used for the stimulation of muscle were according to Ingle(3). The animals were anesthetized with phenobarbital sodium and cyclopal sodium and were subjected to the work test immediately after removal of the adrenal glands and the kidneys. A Nerve Stimulator, Model B, Upjohn, was used to deliver 5 pulses per second. The duration of each pulse was 20 msec. and the intensity was 20 ma. The distance the weight was lifted was recorded on automatic work adders. Each recorder revolution represented approximately 400 g-cm of work. Stimulation was continued until muscular responsiveness was lost. During the work test, the animals were enclosed in a cabinet with the temperature constant at 28° ± 0.5°C. The steroids were dissolved in peanut oil and were given by intermittent subcutaneous injection at the beginning of stimulation and 6, 24, 30, 48, etc. hours later. Each animal received continuous subcutaneous injections of 0.9% sodium chloride solution at the rate of 20 cc per 24 hours per rat. The liver glycogen deposition tests were carried out in the Endocrinology Department of the Upjohn Research Division by Mr. D. F. Kiel. The procedure has been described by Pabst, Sheppard, and Kuizenga(4).

Experiments and results. The data on dosages, numbers of animals and work performance are summarized in Table I. The work response elicited by cortisone acetate

* We wish to express our appreciation to Dr. E. C. Kendall, The Mayo Foundation, Rochester, Minnesota, who supplied the diene acetate; to Dr. Augustus Gibson, The Medical Division of Merck and Co., who supplied the cortisone acetate; and to Mr. D. F. Kiel, Endocrinology Department, The Upjohn Research Division, who performed the bioassays of these two compounds by the liver glycogen deposition test.

1. Kendall, E. C., *Fed. Proc.*, 1950, v9, 501.
2. Higgins, G. M., Woods, K. A., and Kendall, E. C., *Endocrinol.*, 1951, v48, 175.
3. Ingle, D. J., *Endocrinol.*, 1944, v34, 191.
4. Pabst, M. L., Sheppard, R., and Kuizenga, M. H., *Endocrinol.*, 1947, v41, 55.