This work was supported in part by grants from the Life Insurance Medical Research Fund and the National Science Foundation.

Note added in proof. Since this communication was submitted, we have seen a paper by P. J. Bentley (*J. Endocrin.*, **18**, 327; 1959), who observed a similar interaction between calcium and 'Pitressin' on toad bladder.

G. WHITTEMBURY*
N. SUGINO†
A. K. SOLOMON
Harvard Medical School, Boston, Mass.

* Helen Hay Whitney Foundation Fellow. On leave of absence from Facultad de Medicina, Lima, Peru.
† On leave of absence from Nihon University Medical School, Tokyo, Japan.

[1] Staverman, A. J., *Rec. Trav. Chim.*, **74**, 344 (1951).
[2] Paganelli, C. V., and Solomon, A. K., *J. Gen. Physiol.*, **41**, 259 (1957).
[3] Ussing, H. H., "Colston Papers", **7**, 33 (Butterworths Scientific Publications, London, 1954).
[4] Sawyer, W. H., *Endocrinol.*, **66**, 112 (1960).
[5] Hays, R. M., Lamdin, E., Maffly, R. H., and Leaf, A., *Fed. Proc.*, **18**, 66 (1959).
[6] Fukuda, T., *J. Cell. and Comp. Physiol.*, **7**, 301 (1936).
[7] Bozler, E., *Amer. J. Physiol.*, **197**, 505 (1959).
[8] Schultz, S. G., and Solomon, A. K., Fourth Ann. Meeting of the Biophysical Society, Abstract *F4* (1960).

HÆMATOLOGY

MNSs Gene Frequencies in English Blood Donors

ESTIMATES of the MNSs gene frequencies in the English population have been based hitherto on the results of tests with anti-M, -N and -S only[1,2]. Recently, a supply of the very rare anti-s was made available through the kindness of Dr. J. O'Riordan of Dublin[3], and a random series of 1,000 Sutton blood donors was tested with all four antisera. The samples were also tested with anti-Mi^a, -Vw and -Mg. All were negative with the last two antisera, but one donor was found to be *Ms/Ns*, Mi(a +) Vw−, and a study of her family showed Mi^a to be travelling with *Ns*. The results of the major grouping tests are given in Table 1.

Table 1. SAMPLES FROM 1,000 UNSELECTED SUTTON BLOOD DONORS TESTED WITH ANTI-M, -N, -S and -s

Reactions with anti				Number
-M	-N	-S	-s	
+	−	+	−	57
+	−	+	+	140
+	−	−	+	101
+	+	+	−	39
+	+	+	+	224
+	+	−	+	226
−	+	+	−	3
−	+	+	+	54
−	+	−	+	156
			Total	1,000

Table 2. PERCENTAGE FREQUENCIES OF THE MNSs GENES IN ENGLISH PEOPLE

	Sutton series, 1959 Calculations based on the use of Anti-M,-N,-S-s	Sutton series, 1959 Calculations based on the use of Anti-M,-N,-S	Race and Sanger (ref. 1) 1950	Ikin *et al.* (ref. 2) 1952
MS	23·11±1·022	23·58±1·116	24·72	24·02
Ms	30·90±1·102	30·67±1·185	28·31	29·71
NS	7·09±0·678	6·89±0·807	8·02	5·64
Ns	38·90±1·155	38·86±1·233	38·95	40·63
No. tested	1,000		1,419	1,166

Gene frequencies have been calculated by the method of maximum likelihood estimation, and are presented in Table 2. They have also been calculated from the same data, assuming the use of anti-M, -N and -S only; these results are included in Table 2 for comparison along with those of Race and Sanger[1], and of Ikin *et al.*[2], whose tests were in fact made with the three sera only. The calculated gene frequencies, when recombined, give expected figures for the 9 phenotype classes which fit very closely with those observed ($\chi^2 = 3{\cdot}64945$ for 4 d.f.)

I am indebted to Dr. J. H. Edwards of the Medical Research Council Population Genetics Research Unit, Oxford, for the statistical treatment. He has estimated from these results that tests on 800 samples with all four antisera may be expected, on average, to yield as much information as tests on 1,000 samples with the three antisera more readily available.

T. E. CLEGHORN
South London Transfusion Centre,
38 Stanley Road, Sutton, Surrey.

[1] Race, R. R., and Sanger, R., "Blood Groups in Man", 51 (1950); 62 (1954); 74 (1958) (Oxford University Press).
[2] Ikin, E. W., *et al.*, cited by Mourant, A. E., in "The Distribution of the Human Blood Groups", 358 (Oxford University Press, 1954).
[3] O'Riordan, J., and Cann, J., *Vox Sang.* (N.S.), **4**, 242 (1959).

Chemical Constitution of the Red Blood Cell Membrane

SOME authors[1–6] claim that the red blood cell membrane is a continuous lipoid. Others[7–10] do not accept this. They assert that the membrane consists of a protein skeleton with lipoid molecules in the hollows of the protein skeleton.

The work reported here aims at settling this with the help of the Sanger dinitrofluorobenzoic acid method for amino end-group determination[11].

Red cells freed from native protein and its fraction were used. Fractionation was as follows: to 10 ml. red cell suspension 20 ml. physiological salt solution and 10 ml. ether were added. This was shaken for 5 min. and then centrifuged. Three phases resulted: the central layer (the stroma) was solid; the upper ether phase contained lipoid; the lower solution contained hæmoglobin. The extraction was repeated five times. We combined the fraction obtained in the course of the extractions. The stroma and the watery solution from ether were dried and the ether solution evaporated. Thus we obtained ether-soluble lipoid- and hæmoglobin-free stroma, ether hæmolysate and such lipoid fraction as contained any ether-soluble fat-like matter.

Since the alcoholic solution of dinitrofluorobenzoic acid causes hæmolysis on native red cells, the method had to be modified. The reactions were performed with native red cells and aqueous solution, with physiological salt of dinitrofluorobenzoic acid at *p*H 8·0 (0·15 *M* HCO_3–CO_3), at 37° C. by slight stirring for 60 min. Hydrolysis of the products was effected with 6 *N* hydrochloric acid at 105° C. (6 hr.). Analysis of purified dinitrophenyl derivatives was performed with paper chromatography[12].

The chromatogram of native red cell and of its three fractions is illustrated in Fig. 1.

Each substance was identified by its mobility and by the application of model compounds.

On the surface of the native red cell[9] two amino-acids, arginine and serine amino end-groups, are to be found. Serine can be derived both from stromatin-proteins and from cephalin; consequently its presence is not conclusive. The arginine, however, is exclusively of protein origin, therefore the stroma-proteins are not likely to be encircled by coherent lipoid membrane. This fact is confirmed by Block and Bollding[13], who