develop. Cross-resistance to neomycin was demonstrated in the strains of staphylococci which had increased in resistance to streptomycin following subcultures with the latter agent used either alone or in combination with erythromycin; such cross-resistance to neomycin did not occur in the strains which had become resistant to erythromycin following exposures to that antibiotic alone. The biological characteristics of the staphylococci, including coagulase production, remained unaltered following the development of resistance to either streptomycin or erythromycin, except for decreased production of pigment and less active growth of the resistant strains. The development of resistance to both erythromycin and streptomycin was delayed and depressed when the organisms were repeatedly subcultured in the combination of the 2 antibiotics. 3. It is concluded that the development of resistance to penicillin, streptomycin or erythromycin *in vitro* may be delayed and depressed by the use of a combination of erythromycin with either penicillin or streptomycin.

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Chloride Exchange between Human Erythrocytes and Plasma Studied with Cl^{36}.* (20043)

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It has been demonstrated by the use of isotope technics that in human blood sodium and potassium constantly exchange between erythrocytes and plasma under simulated physiologic conditions *in vitro* (1-6). This exchange is partially controlled by chemical processes which are very susceptible to temperature change and to some metabolic poisons. With this in mind, the exchange of chloride between plasma and erythrocytes has been investigated to determine whether the presence of similar metabolic control of the exchange of this anion could be demonstrated. In these experiments, Cl^{36} was added to whole blood, and the influence of changes in temperature and of several enzyme inhibitors on the speed of chloride uptake by the red cells measured. The inhibitors tested were NaCN, $NaNO_2$, Na-mono-iodoacetate, NaF, $HgCl_2$, Na-pyrophosphate, and sulfanilamide.

Methods. Heparinized venous blood was obtained from healthy donors, and 10 ml placed in paraffined flasks exposed to air at room temperature. After 30 minutes or longer, Cl^{36}, in 0.04 ml of saline solution, was

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TABLE I. Results of All Measurements of Chloride Exchange between Human Erythrocytes and Plasma.

Exp. No.	Temp., °C	Conc. of added compounds	Duration of exp. Min.	Duration of exp. Sec.	Specific activity (Cl^{36}) of plasma as % of whole blood specific activity
I	23	0	5		96.2
			12		99.9
			20		94.2
			40		99.2
II	21	0	2	20	92.8
III a	38	0	2	35	102.2
b	"	0	3	7	101.9
c	"	NaCN 10^{-3}M	2	17	94.7
d	"	$NaNO_2$ 1.5×10^{-2}M	2	27	95.2
e	"	Na-mono-iodoacetate 10^{-3}M	2	25	95.4
f	"	NaF 10^{-2}M	2	20	94.8
g	"	$HgCl_2$ 10^{-3}M	2	30	100.3
h	"	Na-pyrophosphate 10^{-3}M	2	38	98.7
i	"	Sulfanilamide 2×10^{-2}M	2	35	96.1
IV	2–6	0	2	38	98.2
				Mean	97.3

added to the blood and mixing achieved manually by vigorous rotation of the flask for 30 seconds. Enzyme inhibitors were added 5-10 minutes before the isotope. After mixing, 5 ml of blood was removed and centrifuged briefly so that plasma could be separated approximately 2.5 minutes after adding Cl^{36} to the blood, and its radioactivity measured by methods previously described(7). After 3-4 minutes of additional centrifugation, plasma for chloride determination by the Schales technic(8) was obtained. The remaining whole blood in the flask was hemolyzed by freezing and similarly analyzed for radioactivity and chloride content. When the specific activity of the plasma had fallen to that of the whole blood, sufficient chloride must have moved in and out of the red cells to raise their specific activity to that of the plasma. Using formulations discussed in the literature(1), it was possible to estimate the lower limit of chloride transfer necessary to achieve this state. Since in all experiments there was no detectable difference in whole blood and plasma specific activity by the time they were first measured, at least 4 (probably more) $T\frac{1}{2}$ periods(1) were considered to have elapsed from the time of adding Cl^{36} until separation of the plasma.

Results. The results of all experiments and the conditions under which chloride exchange was measured are summarized in Table I. In Exp. III-c, plasma was collected most rapidly of all experiments and showed essentially the same specific activity as the whole blood. Calculation of the minimal chloride exchange rate in this instance showed that at least 95 g of chloride entered per liter of red cells per hour, or an amount equal to 50 times the red cell chloride content. In the several experiments, the exchange was not detectably different at 38°C, 21°C, or 2° to 6°C. Also, the addition of enzyme inhibitors and the conversion of hemoglobin to methemoglobin with sodium nitrite did not detectably alter chloride exchange.

Discussion. These data are in agreement with the qualitative results of others(9,10) and indicate that the rate of exchange of chloride between red cells and plasma is at least 600 and 1300 times more rapid than that of sodium and potassium respectively. The true difference may be very much greater, but the conditions of these experiments offered technical difficulties which made it impossible to measure the true speed of chloride exchange. In view of the ability of the red cells to affect the "chloride shift," the access of chloride to the cytoplasm of these cells may be controlled by conditions different from those

in other body cells. An illustration of such a difference between cells is given by the unusual ability of those of the ileum and kidney to absorb chloride against concentration gradients. While this process may not involve control of chloride transfer directly, it is associated with metabolic processes that can be inhibited by added chemical compounds (11,12).

The experiments reported here do not suggest the presence of any metabolic processes controlling the exchange of chloride between erythrocytes and plasma, but they do not eliminate this possibility. They are consistent with the hypothesis that chloride enters red cells by passive diffusion. Approximate calculations show that the thickness of the plasma layer surrounding each erythrocyte in whole blood is about one micron, and that the red cell surface area in the 10 ml samples of blood used totals approximately 6 m^2, so that ample opportunity for rapid diffusion exists. However, because the actual rate of chloride transfer could not be measured, and because all possible metabolic systems were not inhibited, the results are not conclusive.

Summary. The rate of transfer of chloride into and out of human erythrocytes was estimated *in vitro* with Cl^{36} and found to be at least 600 to 1300 times greater than that of sodium and potassium respectively. Incubation at low temperature, the conversion of hemoglobin to methemoglobin, and the addition of metabolic poisons, were all without detectable effect on the rate of transfer. These data are compatible with the hypothesis that chloride enters erythrocytes by passive diffusion, but they do not eliminate the possibility that metabolic processes responsible for Cl transfer may exist which are capable of transporting chloride into and out of the red cell at a very rapid rate.

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A Pneumotropic Virus Isolated from C_3H Mice Carrying the Bittner Milk Agent. (20044)

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In a preliminary report(1), account was given of a virus isolated from C_3H mice known to carry the Bittner Milk Agent. This virus kills young suckling mice although inoculation of older suckling and of adult mice results in no obvious disease. Pathologic findings, as fully described elsewhere(2) are limited largely to the lungs regardless how the virus is inoculated and are different from those encountered in other types of mouse pneumonitis known to us. They consist primarily of swelling and proliferation of endothelial cells lining small blood vessels. The nuclei are swollen and contain inclusion bodies staining

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