ture; the protein was inferred to be the membrane "structural protein" of Green and his colleagues (*6*, *7*). This inference has found support in the recent work of Steim and Fleischer (*5*) on the structural protein of mitochondria.

In connection with these data, we proposed a new model for the general structural organization of the components of membranes (*1*). Wallach and Zahler (*3*) independently proposed a model that is similar in many respects. In this model, the membrane components are held together predominantly by noncovalent hydrophobic interactions, as had been suggested earlier (*6*, *8*). The ionic and polar heads of the phospholipids, together with the charged groups of the proteins, are all situated at the exterior surfaces of the membranes in contact with the bulk aqueous phase. The interior of the membrane contains the hydrophobic tails of the phospholipids, the rest of the protein, and other hydrophobic components, such as cholesterol. By contrast, membrane models of the Davson-Danielli-Robertson type (*9*) have the ionic and polar heads of the phospholipids submerged under a monolayer of protein on both surfaces of the membrane, the entire structure held together predominantly by electrostatic interactions between the ionic heads of the phospholipids and the charged groups of the protein monolayers.

We now report some results of the modification of red blood cell membranes with preparations of phospholipase C. This enzyme specifically catalyzes the hydrolysis of phospholipids to diglycerides and water-soluble phosphorylated amines (*10*). Human red blood cell membranes were prepared by the method of Dodge *et al.* (*11*), as in our previous studies (*1*). The intact membranes were then thoroughly dialyzed into a buffer containing 5 m*M* imidazole, 0.1*M* KCl, and 2 m*M* $CaCl_2$ at *p*H 7.3. A commercial preparation of phospholipase C from *Clostridium welchii* (*12*) was added to membranes at a concentration corresponding to about 1 unit per milligram of membrane protein. The mixture was incubated at 37°C and the extent of reaction was followed by measuring the phosphorus released into the supernatant after dilution and centrifugation of the membranes for 60 minutes at 30,000*g*. Phosphorus was assayed by the procedure of Bartlett (*13*).

Under these conditions 68 to 74 percent of the total membrane phosphorus was released into the supernatant within the first 10 minutes of the reaction. Since by far the largest part of the membrane phosphorus is in the form of phospholipids (see *14*), this figure represents the amount of phospholipid that was hydrolyzed. Neither longer incubation times nor a second addition of enzyme raised this value. The supernatant did not contain detectable amounts of cholesterol (*15*) or of carboxylic acid esters (*16*), which indicates that lipid components and diglycerides were not released from the membrane by treatment with phospholipase C. The absorbance at 280 mμ of the supernatant after treatment was identical with that of an untreated control, showing that protein was not released from the membrane. The membranes remained intact when viewed in the phase microscope; no electron microscopic observations were made.

The circular dichroism spectrum of the membranes treated with prosopholipase C was indistinguishable from that of an untreated control (*1*); the minimum at 224 mμ and the smaller minimum at 210 mμ were unchanged in either magnitude or position by action of the enzyme (*17*).

These results indicate that rapid cleavage and release of a major fraction of the ionic heads of the phospholipids occur upon the action of phospholipase C on intact cell membranes, without disruption of the membrane or alteration of the overall conformation of the protein in the membrane. This suggests that: (i) the phosphoester bonds that are hydrolyzed are readily accessible to the phospholipase C molecule in the intact membrane; and (ii) electrostatic interactions between phospholipids and membrane proteins play only a secondary role, presumably to hydrophobic interactions, in maintaining the integrity of the membranes and in determining the conformation of the membrane proteins. These data are, therefore, more consistent with the model of membrane structure we have proposed (*1*) than with the Davson-Danielli-Robertson (*9*) model.

JOHN LENARD*
S. J. SINGER
Department of Biology, University of California, La Jolla 92037

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17. By contrast, the action of phospholipase A on red blood cell membranes (B. Brandhorst, J. Lenard, S. J. Singer, unpublished observations), which converts lecithin to lysolecithin, produces a fragmentation of the membrane and a shift of the circular dichroism spectrum toward the ultraviolet, much as treatment with detergent does (*1*).
18. Supported by grant AI-04225 from the National Institutes of Health. J.L. was an advanced research fellow of the American Heart Association, 1965–67.
* Present address: Department of Biochemistry, Albert Einstein College of Medicine, New York 10461.

12 January 1968

Genetic Selection for Voluntary Alcohol Consumption in the Albino Rat

Abstract. *By outbreeding Wistar rats and selecting for breeding animals that differ in their alcohol consumption, we have raised two genetically different lines. Marked differences between the sexes and the strains were evident by the eighth generation. Selection is reflected in the regression coefficient .754, which accounts for 65.9 percent of the variance. The heritabilities differ significantly in the two sexes,* h^2 *for the males being .263, and for the females .371; this difference seems mainly ascribable to sex-linkage of some of the genetic factors controlling voluntary consumption of alcohol.*

The underlying assumption in experiments on voluntary selection of alcohol is that heritable biochemical reactions which control physiological mechanisms also to some extent regulate alcohol consumption. This hypothesis has been the basis of numerous animal experiments used to clarify the heritability of voluntary consumption of alcohol. Reed (*1*) investigated the selection of alcohol by six different inbred lines of rats, and, although the investigation was not designed for genetical research, the results reveal differences between the strains. Mardones (*2*) set out to raise two inbred rat strains that would differ in their alcohol habits; he found the differences to be genetically determined, although

the heritability was fairly low. These strains differed relatively little, however, and both must be considered comparatively moderate consumers of alcohol; moreover, individual differences in alcohol consumption were also small, ranging from 0.02 to 0.72 ml/100 g of absolute alcohol. That the original stock was apparently very homogeneous probably accounts for the lack of differences correlated with alcohol consumption between the sexes, and between the strains (*3*). Rodgers and McClearn (*4, 5*) have studied the voluntary consumption of alcohol by mouse strains inbred for long periods, and have associated the differences found with metabolic data. But the mouse strains used had been selected for traits other than alcohol consumption, and they differed from each other considerably in many other respects; thus interpretation of the results is difficult. Apparently for that reason, the authors avoided quantitative estimation of heritability and contented themselves with the observation that different mouse strains differ widely in the voluntary consumption of alcohol. Because several years of cross-breeding our Wistar rat strain resulted in a very wide range of individual variation and in a rather high average preference for alcohol (*6*), we started to raise two rat strains which would differ genetically in their alcohol preference.

To avoid restriction of individual variation at a given level, we at first intentionally avoided mating sibs. The first four outbred generations constituted a single line, and from the fifth generation on, there was an alternation of sib-mating and outbreeding. The purpose of this slow selection was to secure the maximum possible difference between strains. Inasmuch as previous work (*1–5*) warranted the assumption that heritability is rather low, the breeding animals were chosen in the manner proposed by Falconer (*7*), by selection within the family so that about 25 percent of the animals were used for continued breeding.

Every animal in each generation was tested as follows. After puberty (at 4 months) the animals were isolated in individual cages, where for 10 days the only available fluid was a 10 percent (by volume) alcohol solution. After this period, the animals were given access to water as well as to the alcohol solution for 4 weeks during which the mutual location of the tubes was changed once a week; the rats were provided free access to standard laboratory food. The quantities of water and alcohol solution consumed were measured daily, food consumption was reckoned once a week, and the rats were weighed weekly. As previously shown (*8*), fluid intake may vary greatly in animals consuming the same amount of alcohol per unit of body weight. For this reason, alcohol intake per body weight was chosen as the phenotypic measure; this is reduced to milliliters of absolute alcohol per 100 g of body weight per day. Fuller (*9*) criticizes the use of only one alcohol concentration for determination of the phenotype because inbred mouse strains differ in their concentration preferences. According to our experience, rats consume the same amount of absolute alcohol when offered the choice between water and solutions of alcohol (5 to 15 percent by volume). Rick and Wilson (*10*) have reported the same observation. On this basis, one alcohol concentration can be used to establish the phenotype.

As breeding has been selective, estimation of the classic heritability value h^2 from the regression coefficient between the mean of parents and the values of the offspring (mid-parent/offspring regression) is misleading. In Fisher's opinion (*11*), however, a value that measures the effectiveness of selection can be calculated in this way. The value derived from the regression of the offspring to their F_7 parents was .754 $\pm$ .15 and is highly significant ($t = 4.32$, $P < .001$). The model accounts for 65.9 percent of the variance in the data. A phenomenon to be noted in all generations tested so far is that females consume markedly more alcohol per unit of body weight than males do as has been reported (*6*). Although the means of the sexes differ, the coefficients of variation are similar. To obtain a rough estimate of heritability, we made 14 F_2 crosses in a manner permitting calculation of the h^2 value. Comparison of the means of the F_1 generation with the means of the two parental strains provide evidence that the genetic factors are, for the most part, additive in their effect. The mid-parent/offspring regression for the males, .263 $\pm$ .06 ($t = 4.12$, $P < .005$), was lower than the corresponding value for the females, .371 $\pm$ .09 ($t = 3.40$, $P < .01$). The higher heritability value of the females seems mainly ascribable to sex-linked inheritance. Rodgers and McClearn (*4*) likewise reported that the influence of the females is greater. They were not able to demonstrate maternal effect when they removed the young; neither was I. That the sexes differ with respect to the rate of alcohol elimination seems at least partly to explain the difference in preferences although this difference only appears in strains where alcohol consumption is high.

In eight generations selection has resulted in a curve of variation, with four peaks, constituted by the females and the males of the two strains (Table 1). Furthermore, even with due allowance for sex, the two strains differ in weight, drinkers being lighter than nondrinkers. Mardones (*3*) observed a similar difference in his strains, although it was not statistically significant. The calorie in-

Table 1. The average values and standard deviations of the functions measured during a 4-week period in drinker and nondrinker strains in the F_8 generation. Statistical values of t and P also shown.

Subjects	Number	Mean weight of rats	Absolute alcohol consumption (ml/100 g body weight per day)	Alcohol preference (% total fluids)	Total calories/ 100 g body weight per day	Total fluids (ml/100 g body weight per day)
Nondrinkers, males	49	363.7 ± 35.4	0.18 ± .13	22.4 ± 16.5	17.6 ± 1.3	8.4 ± 1.1
Nondrinkers, females	42	221.6 ± 16.0	.29 ± .26	29.3 ± 22.7	22.3 ± 1.3	10.6 ± 2.1
Drinkers, males	59	329.6 ± 29.7	.48 ± .25	51.2 ± 24.6	19.0 ± 1.3	9.0 ± 1.1
Drinkers, females	60	207.1 ± 15.3	.97 ± .34	75.3 ± 22.3	24.1 ± 1.4	12.5 ± 2.4
		Statistical validity between groups				
	df	*t* *P*	*t* *P*	*t* *P*	*t* *P*	*t* *P*
Nondrinkers, males–females	89	24.9 <.001	2.4 <.025	1.6 <.200	17.4 <.001	6.1 <.001
Drinkers, males–females	117	28.5 <.001	8.9 <.001	5.5 <.001	20.4 <.001	10.3 <.001
Males, drinkers–nondrinkers	106	5.9 <.001	7.9 <.001	7.2 <.001	5.6 <.001	2.9 <.005
Females, drinkers–nondrinkers	100	4.5 <.001	11.3 <.001	9.9 <.001	6.7 <.001	4.2 <.001

Table 2. The correlation coefficients (r) between functions measured in the males of the drinker strain in the F_7 generation. The limit value of the statistical significance at $P = .05$ is .25; at $P = .01$ it is .32. Functions measured are absolute alcohol and total fluids in milliliters per 100 g of body weight per day, total calories per 100 g of body weight per day, weight at beginning of period, and growth in grams.

Functions	1	2	3	4	5
1 Absolute alcohol	1.00				
2 Total fluids	.65	1.00			
3 Total calories	.43	.58	1.00		
4 Weight	− .19	− .26	− .43	1.00	
5 Growth	.34	− .17	.16	.24	1.00

take of the drinkers is significantly higher than that of the nondrinkers. The same applies to fluid intake. The greater fluid intake is probably due to the diuretic effect of alcohol. And with the same standard food, greater consumption of food usually leads to higher fluid intake; this may reflect a metabolic difference between strains. With respect to weight, such a marked difference between the strains may also signify an inherited metabolic difference, which is associated with alcohol consumption (*6*). The sexes differ in the rate of alcohol elimination, which is higher in females (*6*). Subsequent experiments have indicated that these strains do not differ in the rate of alcohol elimination or in alcohol dehydrogenase activity. The difference between the sexes which is conspicuous in the drinker strain, is not so apparent in the nondrinker strain. In both strains the difference between sexes is greater for alcohol consumption than for alcohol preference. The alcohol consumption as a percentage of total fluid intake has generally been adopted as a measure of preference. In my experiments, the regression of preference values of alcohol intake per unit weight was determined for the F_7 generation, of which there were 210 animals. The regression model accounted for as much as 94 percent of the variance, the regression coefficient being .85. The two measures show a high correlation, but the preference value is at a disadvantage, because it does not vary freely but depends on total fluid intake. From the same data, the correlations of other variables with alcohol consumption were also calculated. Because the four-peaked curve of variation does not permit analysis of the total data, I have chosen drinker males as an example (Table 2), especially because their range of intake was wide but the range of other variables, within individuals, was slight during the experimental period. Consequently, the possible association of some variables with alcohol consumption is easier to discern. The results confirm the validity of the sex and interstrain differences (Table 1), in that calorie intake, fluid intake, and alcohol consumption constitute a positively correlated group of variables. In contrast, the interstrain difference in initial weight shows only a weak negative correlation with alcohol consumption, and the difference is possibly fortuitous.

Rodgers and McClearn (*4*) have adopted the working hypothesis that alcohol consumption is an additively inherited polygenic trait. However, they have not constructed a heritability model, although they have shown that females exert a greater effect on the offspring. Construction of a heritability model on the basis of the hypothesis of self-selection requires that the inheritance be additive, that there be no dominance, that the quantitative effects of the genetic units involved be approximately equal, and that there be no linkage. The experiments of Reed (*1*) reveal large deviation within inbred strains with regard to drinking behavior. These results suggest that the role of inheritance is slight, either because of an inadequate phenotypic measure or of a predominating role of the environment. The model constructed here shows the importance of genetic factors in determining selection of alcohol, although the determining power of the environment still seems stronger. The model will undoubtedly be improved by an elaboration of measuring techniques.

KALERVO ERIKSSON

Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland

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10 October 1967

Hemoglobin Rainier (β^{145} Tyrosine → Histidine): Alkali-Resistant Hemoglobin with Increased Oxygen Affinity

Abstract. *Hemoglobin Rainier, a new hemoglobin variant associated with erythrocytosis, was found in six members of a Caucasian family. Structurally, it represents substitution of histidine for the invariant residue H23 tyrosine in the β-hemoglobin polypeptide chain (β^{145} tyrosine → histidine). Hemoglobin Rainier is the first example of a single amino acid substitution in adult human hemoglobin, causing increased resistance to alkali denaturation.*

Thus far, more than 50 examples of chemical abnormalities in the human hemoglobin polypeptide chains have been reported. For the most part, they represent single amino acid substitutions which usually have no measurable effect on the physiological property of the hemoglobin molecule as a respiratory pigment. Recently, abnormal hemoglobins with a functional abnormality consisting of increased oxygen affinity have been reported (*1, 2*). In heterozygotes for these abnormal hemoglobins, lower volumes of oxygen per red cell are released to the tissue. The consequent relative tissue hypoxia stimulates erythropoietin secretion, producing erythrocytosis. We report the biochemical findings of a new abnormal hemoglobin, associated with increased affinity to oxygen, designated as hemoglobin Rainier.

Hemoglobin Rainier was detected in a 47-year-old Caucasian female with erythrocytosis (hematocrits 57 to 59 percent) of unknown etiology and high normal values of urinary erythropoietin. Five other members of her family had this abnormal hemoglobin accompanying erythrocytosis. Hemoglobin-oxygen dissociation of blood samples from individuals with hemoglobin Rainier indicated increased affinity of hemoglobin to oxygen and decreased heme-heme interaction ($n = 1.2$). the pH of red cells and the Bohr effect were normal (*3*).