U, like other blood factors, is inherited as a simple Mendelian dominant. An attempt was made to study relatives of the rare individuals of type uu, in order to verify this hypothesis. As is shown in Table 3, we were able to examine two complete families, and in two other cases we tested the daughter and brother of the propositus. In families 1, 3, and 4 of Table 3, the other members of the family all proved to belong to type U. In family 2, the wife belonged to type U, but two of the three children belonged to type uu. The occurrence of two additional individuals of the rare type uu in such a short series of families confirms the hereditary nature of this blood factor. Presumably, the factor is inherited by a pair of allelic genes *U* and *u*, where gene *U* determines the presence of the factor and gene *u* its absence. Thus, type uu individuals are always homozygous (genotype *uu*), while type U individuals may be homozygous (genotype *UU*) or heterozygous (genotype *Uu*). The fact that in family 4, both children belong to type U indicates that gene *U* is dominant to gene *u*. In family 2, the type U parent is evidently heterozygous, accounting for the occurrence of type uu in the children.

From the distribution of the types, the gene frequencies are readily calculated. In Caucasoids, gene *u* is apparently absent, or at least very rare. Since the frequency of type uu in Negroids is 1.21 percent, the frequency of gene $u = \sqrt{\text{type uu}} = \sqrt{0.0121} = 11$ percent. Therefore, the frequency of gene $U = 89$ percent.

From these values, the frequencies of the three genotypes among Negroids can be calculated as follows:

genotype $UU = (U)^2 = 79.2$ percent,
genotype $Uu = 2(U)(u) = 19.6$ percent,
genotype $uu = (u)^2 = 1.2$ percent.

Thus, approximately one-fifth of type U individuals among Negroids are heterozygous, and it is not surprising that we encountered such a family in our short genetic study. Moreover, if there is a blood factor u corresponding to gene *u*, the expected frequency of the factor in Negroids would be 20.8 percent. As has already been pointed out, the results obtained by Chalmers, *et al.*, for the Hunter factor (21.7 percent) are remarkably close to this value. Moreover, the fact that all the type uu individuals found to date belong to type N or type MN also suggests a relationship to the Hunter factor, since one of us had previously found (*7*) that blood specimens containing the Hunter factor belonged to type N or type MN. However, as has already been pointed out, the fact that a type uu blood failed to react with anti-Hu serum is evidence that Hunter is not an alternate of U.

Since factor U proves to be important for the selection of donors for transfusion to Negroids, a source of specific antiserum is desirable. We had available numerous immune rabbit serums prepared by injections of human blood in order to produce anti-M and anti-N serums. Since all the rabbits had almost certainly received blood of type U, the possibility existed that the antiserum might have U antibodies. Accordingly, 11 different serums were absorbed with type uu blood in order to remove the human species-specific antibodies. Tests after absorption failed to reveal the presence of any U antibodies, however. Experiments are now in progress in which one of the type uu individuals is being immunized by injections of type U blood in order to produce typing serum.

Note added in proof: After four monthly injections, there is still no evidence of specific antibody formation.

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Effect of Synthetic Thioctic or Alpha Lipoic Acid on the Voluntary Alcohol Intake of Rats

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It has been shown that a proportion of rats fed a diet containing only 11 pure vitamins as a source of the vitamin-B complex increase their voluntary alcohol intake, and that a large proportion of these rats decrease their intake when they receive a supplement of liver or yeast (*1*). The substance present in liver and yeast responsible for this effect has been called factor N_1 (*2*).

On the other hand, it has been shown that some microorganisms utilize pyruvate only when the medium contains a factor that has been isolated and synthetized and given the names of thioctic acid (*3*) and alpha lipoic acid (*4, 5*).

Since we have assumed, as a working hypothesis, that the increased voluntary alcohol intake observed in the aforementioned experimental conditions could be the consequence of a slight disturbance in the carbohydrate metabolism at a step higher than the C_2 compounds, it seems desirable to study the effect of this synthetic substance on the voluntary alcohol intake of rats depleted of factor N_1 (*6*).

The alpha lipoic or thioctic acid was given to each experimental rat during a period of 4 to 10 days, after at least 90 days of feeding the aforementioned diet and after voluntary alcohol intake was sufficiently stabilized. The alcohol intake was measured daily, and the average for the 11th to 20th days after the first

dose of thioctic acid or alpha lipoic acid was compared with that of the 10 days preceding the supplement.

There is a proportion of rats in our colony that do not show a decrease of the alcohol intake after liver supplement. Unfortunately, we cannot select them before starting the experiment. For this reason, we have to separate them afterward, testing the effectiveness of liver in each of the experimental rats. For this purpose, the rats received dry liver in doses of 2 g per 100 g body weight per day during 10 days, and the alcohol intake of this new period was also measured. Obviously, we considered in this study only the results obtained in the rats that decrease their alcohol intake after liver supplement.

factor N_1 exhibit a slight decrease of the alcohol intake when the diet is supplemented with alpha lipoic or thioctic acid, and that this decrease is significant around the 2-percent level. On the other hand, they show that liver supplement induced a more consistent decrease of the alcohol intake of the same rats.

Since the activity of dry liver is equivalent to 2 to 4 μg per gram (*7–9*), the liver supplement (total dose 20 g per 100 g body weight) represents 40 to 80 μg of thioctic or alpha lipoic acid. Thus, it is clear that there are rats sensitive to liver and nonsensitive to equivalent or higher doses of alpha lipoic or thioctic acid; hence, the effect of liver can be attributed only in part to the thioctic or alpha lipoic acid contained in it. If we consider as factor N_1 any nutrient, differ-

Table 1. Effect of alpha lipoic or thioctic acid and of liver on the voluntary alcohol intake (milliliters per 100 g body weight per day) of N_1-depleted rats.

	Line	Range		Arithmetic mean	Standard deviation	t*
		Lower	Higher			
Experimental: 31 rats (17 males and 14 females)						
10 days before supplement (*A*)	1	0.28	1.02	0.56 ± 0.031	0.17 ± 0.022	
11th to 20th days after first dose of alpha lipoic or thioctic acid supplement (*B*)	2	.12	0.71	.46 ± .026	.14 ± .018	(1) 2.4
10 days on liver supplement (*C*)	3	.02	.49	.20 ± .025	.11 ± .017	(1) 7.0
A–B	4	−0.21	.41	.11 ± .026	.14 ± .018	
B–C	5	−0.08	.51	.26 ± .020	.15 ± .020	(4) 3.8
Control: 33 rats (15 males and 18 females)						
Same days as *A*	6	0.25	.72	.41 ± .022	.12 ± .016	
Same days as *B*	7	.07	.75	.40 ± .038	.22 ± .027	
Same days as *C*	8	.14	.81	.40 ± .033	.19 ± .023	
A–B	9	−0.29	.55	.01 ± .033	.19 ± .024	(4) 2.4
B–C	10	−0.19	.23	.00 ± .019	.11 ± .013	(5) 7.6

* The figure in parentheses represents the line with which the comparison was made.

On the other hand, sometimes spontaneous fluctuations of alcohol intake occurs in rats. This made it necessary to use a control group composed of rats depleted of factor N_1 and sensitive to liver supplement. The control group did not receive any supplement during the days that the experimental groups were studied.

The experimental rats were divided into four groups of 10 rats each, receiving alpha lipoic or thioctic acid orally in total doses of 62.5, 125, and 750 μg per 100 g body weight, and subcutaneously 62.5 μg per 100 g body weight. Of these 40 rats, only 31 (17 males and 14 females) were sensitive to liver. Since the results did not differ among the groups, all of them were considered as a whole in the statistical analysis. The control group included 33 rats (15 males and 18 females).

Table 1 summarizes the results obtained in the experimental rats and in the controls.

The experimental data show that rats depleted on

ent from the B vitamins known before the discovery of alpha lipoic or thioctic acid, that induces a decrease on the voluntary alcohol intake of deprived rats, we have to conclude that this factor is not a single substance but is composed, at least, of alpha lipoic or thioctic acid and of another substance yet unknown.

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