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Genetic variations in plasma lipid content in mice

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SUMMARY

Large genetic variations in plasma concentrations of total lipids, cholesterol + cholesterol ester, and phospholipids were found in STR/1N, DBA/2JN, and A/LN mice and their crosses. The concentrations were constant from 2 to 16 months of age and were independent of sex. The levels of the three fractions varied in parallel fashion among the various mating groups studied. The data suggest that the genetic factors governing the plasma lipid concentrations were polygenic and had an over-all additive behavior. Inheritance of greater body weight was generally dominant or displayed heterosis. Hepatic total lipid concentrations did not correspond to plasma values in many mating groups. Reciprocal differences in hepatic lipid concentration were found in some of the crosses of STR/1N and A/LN, whereas these were not found for the plasma values.

It has been reported that the serum cholesterol concentration varies in different strains of mice (1, 2). In the present study, it will be demonstrated that there are genetic variations in the content of the several major lipid fractions of the plasma in mice. The patterns of inheritance of factors governing these levels and their relation to certain other genetically influenced aspects of lipid metabolism (body weight, hepatic weight, and lipid content) have been determined in part. In addition, coat color, hepatoma formation, and sacral polymorphism have been analyzed in relation to plasma lipid concentrations. Absence of a relationship of plasma lipids to osteoarthritis in these mice has been noted elsewhere (3).

METHODS AND MATERIALS

Animals and Experimental Conditions. Three inbred parent (P) strains of mice (4) were studied: STR/1N (hereafter designated S), DBA/2JN (D), and A/LN (A). The S mice have high plasma lipid concentrations and become obese on a standard dietary regimen; the A mice are lean and have low lipid values; the D mice also are lean but have lipid concentrations intermediate between the other two strains. F_1 reciprocal hybrids were made between S and A, and between S and D mice. Reciprocal F_2 crosses were made between the F_1 mice, and backcrosses (BC) between the F_1 and the P lines. In each mating group designation, the female parent was listed first. The number of animals in each mating group analyzed is recorded in Table 3; the total number was 645. Only nonbreeder males were examined in this experiment.

The animals received Purina Laboratory Chow. They were killed at 16 months of age. Blood was obtained by cardiac puncture. Some animals were fasted; others were not. The data from fasted mice were analyzed separately from the nonfasted ones. Further details of housing and breeding are presented elsewhere (3).

To establish the validity of using mice 16 months old as representative of the values to be obtained throughout life, 84 additional male mice of the P strains were studied by comparable means at 2 and 10 months of age (Table 1). In addition, lipid concentrations were also determined in 11 female S mice 10 months old (Table 2).

Chemical Procedures. Duplicate samples of heparinized blood were obtained for microhematocrit (5) (Drummond Microhematocrit, Drummond Scientific Company, Philadelphia, Pa.), hemoglobin (6), and glucose (7) determinations. Total lipids were determined by Bragdon's method (8); phospholipids by the Fiske-Subbarow method on the digested aliquot

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TABLE 1. FASTING PLASMA LIPID CONCENTRATIONS (MEAN AND STANDARD ERROR) IN MALE MICE AT VARIOUS AGES

Lipid	Strain	Age (months) 2	10	16
		mg/100 ml		
Total lipids	S	743 ± 55.2 (4)*	724 ± 24.4 (4)	941 ± 72.1 (10)
	D	446 ± 14.2 (5)	505 ± 10.8 (5)	430 ± 13.3 (10)
	A	361 ± 7.4 (5)	398 ± 14.2 (5)	361 ± 14.8 (11)
Phospholipids	S	314 ± 28.8 (4)	331 ± 23.2 (4)	346 ± 30.2 (10)
	D	141 ± 6.6 (5)	173 ± 6.0 (5)	147 ± 14.8 (10)
	A	128 ± 6.4 (5)	150 ± 2.1 (5)	140 ± 6.8 (11)
Cholesterol + cholesterol ester	S	240 ± 12.7 (4)	264 ± 22.5 (4)	288 ± 17.1 (10)
	D	97 ± 3.8 (5)	97 ± 2.7 (5)	151 ± 3.6 (10)
	A	77 ± 1.4 (5)	76 ± 2.0 (5)	109 ± 7.4 (11)

* The figures in parentheses are the number of mice in each group.

(9); free and total cholesterol by the $FeCl_3$ method (10). Cholesterol and cholesterol esters were calculated as suggested by Bragdon (8).

When hepatomas were present the total lipid concentration of the liver was determined on the portion from which the tumor had been excised.

Statistical Analysis. The data were analyzed in three ways.

1. Analysis of variance was used for two purposes: (*a*) to estimate, from the within-litter variances from each mating type, the genetic and environmental contributions to the variation of each of eleven variables (plasma concentration of total lipids, of cholesterol + cholesterol ester, and of phospholipids; body weight at 12 and at 16 months; weight and lipid content of the liver; osteoarthritis scores; femur lengths; hematocrit and hemoglobin concentrations); (*b*) to determine if any of seven discrete methods of grouping the animals (by litter; whether fasted or not fasted; according to 5 types of coat color; whether the mice had osteoarthritis or no arthritis; whether there were 3 or 4 sacral segments; whether the mice had hepatomas or no hepatomas; whether the mice had pulmonary adenomas or no adenomas) had any relationship to or effect on any of the measured variables. The data

TABLE 2. FASTING PLASMA LIPID LEVELS (MEAN AND STANDARD ERROR) OF MALE AND FEMALE STR/1N MICE

Sex	Body Weight	Plasma Lipids Total	Phospholipid	Cholesterol + Cholesterol Ester
	g		*mg/100ml*	
Female* (11)†	45.6 ± 2.1	710 ± 45.0	348 ± 23.0	309 ± 25.4
Male (7)	41.7 ± 1.5	785 ± 36.3	357 ± 19.7	294 ± 21.1

* Virgins and retired breeders, 10 months old.

† The figures in parentheses are the numbers of mice in each group.

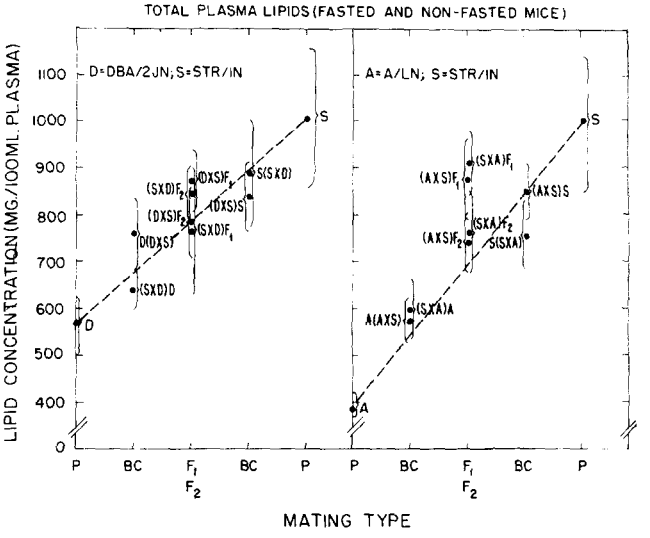

FIG. 1. Total plasma lipid concentrations in various genetic groups. The brackets enclose two standard errors on either side of the means. The dotted line represents the theoretical average that would result if the genes had completely additive effects on the phenotypes (see text).

were regrouped seven times and a one-way analysis of variance performed for each of the seven ways of grouping 11 measured variables and 19 mating types. It is possible that a co-variance analysis might have provided a better estimate of the effects of the variables on each other, but such an extension of the present work has not been undertaken. A summary of the results of the possible 1,463 analyses that contributed information has been recorded elsewhere (3).

2. The mean value and approximate confidence limits of each of the 11 variables was plotted for each

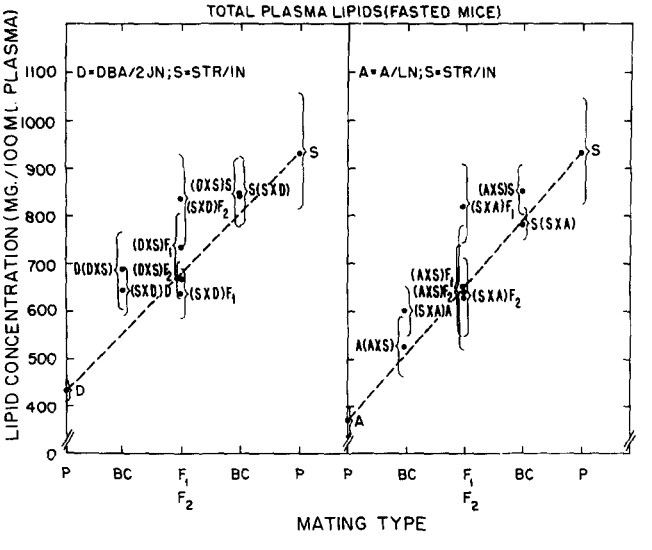

FIG. 2. Mean plasma lipid concentrations in various genetic groups that had been fasted.

TABLE 3. PLASMA LIPID CONCENTRATIONS* IN VARIOUS MATING GROUPS (FASTED AND NONFASTED)

Groups	Total Lipids		Cholesterol + Cholesterol Ester		Phospholipids	
	No. Mice	Concentration	No. Mice	Concentration	No. Mice	Concentration
		mg/100 ml		*mg/100 ml*		*mg/100 ml*
S	19	1009 ± 74.5	20	287 ± 11.8	20	330 ± 19.9
A	21	395 ± 12.8	25	104 ± 3.6	25	138 ± 4.7
(S × A)F_1	27	911 ± 34.9	27	267 ± 11.5	27	316 ± 15.2
(A × S)F_1	31	879 ± 42.3	31	227 ± 10.0	31	286 ± 9.7
(S × A)F_2	48	765 ± 44.7	47	226 ± 11.8	48	266 ± 9.3
(A × S)F_2	52	743 ± 31.3	53	217 ± 11.7	53	271 ± 10.2
A(A × S)	36	573 ± 24.8	36	155 ± 7.1	35	196 ± 7.6
(S × A)A	28	598 ± 32.2	28	197 ± 12.2	28	222 ± 12.1
S(S × A)	27	758 ± 36.1	27	263 ± 9.8	27	285 ± 13.8
(A × S)S	24	852 ± 29.8	24	269 ± 12.4	24	306 ± 11.6
S	19	1009 ± 74.5	20	287 ± 11.8	20	330 ± 19.9
D	27	567 ± 28.3	27	132 ± 4.7	27	187 ± 10.3
(S × D)F_1	35	764 ± 68.4	35	230 ± 12.9	35	267 ± 20.0
(D × S)F_1	38	874 ± 32.7	38	214 ± 6.4	38	286 ± 6.6
(S × D)F_2	58	846 ± 28.9	60	236 ± 8.7	60	291 ± 11.1
(D × S)F_2	60	785 ± 39.2	60	231 ± 9.7	60	269 ± 12.2
D(D × S)	26	759 ± 37.8	28	183 ± 9.4	28	247 ± 16.5
(S × D)D	25	641 ± 21.4	25	195 ± 8.3	25	237 ± 6.9
S(S × D)	26	889 ± 57.5	26	296 ± 20.3	26	337 ± 24.4
(D × S)S	27	838 ± 36.9	26	284 ± 9.8	27	300 ± 12.6

* Mean ± standard error.

generation on the vertical axis of graphs (Fig. 1 and 2), and the mating types were grouped on the horizontal axis by the proportion of nonsex linked material they have in common with the parental (P) generations. The dotted line connecting the P values represents the theoretical average that would result if the parental means represent the true means and the genes had completely additive effects on the phenotypes; i.e., if there were no over-all dominance or recessiveness of the genes and nonrandom environmental influence on the findings.

3. The distribution of the measurements of the 11 variables was determined in each of the mating types to see whether there were multiple modes in the frequency of the values in the F_2 and BC generations, as would be expected if segregation by 1 or 2 genes was taking place in the absence of much environmental variability.

RESULTS

Effect of Age on Plasma Lipid Concentrations. The mean fasting concentrations of phospholipids and total lipids were, in most instances, unchanged within each of the three P strains at 2, 10, and 16 months (Table 1). There was a tendency for the cholesterol + cholesterol ester values to be somewhat higher at 16 months than in the younger groups. Nevertheless, these differences were much smaller than the interstrain differences in each age group.

Effect of Sex on Plasma Lipid Concentrations. No significant sex differences were found in the three fractions in S mice 10 months old (Table 2).

Effect of Fasting. There was a consistent reduction in plasma lipids due to fasting (compare Fig. 1 and 2). The reduction in general was 15–30% of the nonfasted level and was statistically significant in 9 of 13 groups that could be tested (3). The reduction in plasma cholesterol + cholesterol ester and in phospholipids was less consistent and smaller than in total lipid concentrations, suggesting that fasting had its greatest effect on the neutral lipid of the plasma.

Liver weight was consistently reduced by fasting; the differences were significant in nearly all groups of animals. The reduction was in general about 20% of the weight of the liver in nonfasted animals. No consistent changes in the hepatic lipid concentration were found as a result of fasting.

Genetic Variation in Plasma Lipid Concentrations. Variations in each of the lipid fractions were found among the three parental strains (Table 3). The S mice consistently had the highest concentrations of total lipids, cholesterol + cholesterol ester, and phospholipids. The A mice had the lowest mean values.

TABLE 4. MEANS, WITHIN-LITTER VARIANCE, AND COEFFICIENTS OF VARIATION FOR TOTAL PLASMA LIPID CONCENTRATION

Mating Type	No. Mice	Mean Lipid	Within-Litter Variance	Coefficient of Variation
		mg/100 ml		
S	19	1,009	105,507	0.322
A	21	395	3,417	.148
F_1*	58	894	28,016	.188
F_2*	100	754	33,080	.241
BC_A*	64	584	9,974	.171
BC_S	51	802	19,792	.175
S	19	1,009	105,507	.322
D	27	567	21,685	.260
F_1*	73	821	14,689	.148
F_2*	118	815	33,958	.226
BC_D*	51	701	19,993	.202
BS_S*	53	863	32,003	.207

* Based on pooled values of reciprocal crosses. The subscript in the BC designations refers to the parent line to which the back cross was made. The data are from fasted and nonfasted mice.

Inspection of the means of the fasted mice led to no different genetic interpretation than the graph presented for the fasted + nonfasted values (Fig. 1). The analyses presented are based on the data from fasted + nonfasted mice.

Analysis of the variance on total plasma lipid concentration disclosed that small environmental influences (between-litter variances) affected the levels. Genetic control of the variability in total plasma lipids is suggested by the fact that the within-litter variances and coefficients of variation are greater in the F_2 than in the F_1 crosses (Table 4). The BC mice showed more variation than the F_1 in the S × D crosses, as would be expected. However, the fact that the BC mice showed less variation than the F_1 in the S × A crosses does not fit the genetic expectation. The S and D inbred mice unexpectedly showed more variation than the F_1 crosses, perhaps because of the large sampling error of the estimates of these variances or because inbred animals may be less able to cope with the variability of the environment.

The mean values of the total plasma lipids (Fig. 1) are amenable to a more definite genetic interpretation. They followed quite closely the theoretical line indicating additive genetic variation in both crosses (Fig. 1). No consistent differences were found between the reciprocal values.

Little evidence was found in the distribution of total plasma lipid concentrations for multiple modes of distribution in the F_2 and BC groups to suggest that segregation by one or a few genes was taking place. Nevertheless larger samples of data would be necessary to make a definite statement in this regard.

A similar analysis of the cholesterol + cholesterol ester and phospholipid concentrations indicated that the mode of inheritance of these characteristics was similar to that for the total plasma lipids (Fig. 3 and 4).

Hepatic Lipids. The mean hepatic total lipid concentrations in 10 mice of each P strain (fasted and nonfasted) were: S, 65.9 ± 6.7 (*S.E.*) mg/g liver; A, 52.3 ± 1.4; D, 60.6 ± 4.3. The effects of fasting on these values in the various mating groups was quite variable, and no significant trends could be detected on the limited number of samples tested.

Little or no relationship existed between hepatic and plasma concentrations of total lipid. In general, the weight of the livers of the heavier mice was greater than those of lean animals; consequently, the absolute amount of total lipid in the former was greater than in the latter. There was a suggestion of a maternal influence on the hepatic lipid concentration in A × S crosses, although not in the D × S: offspring of A mothers had somewhat lower concentrations than did the reciprocals.

Body Weight and Plasma Lipids. The body weight of S mice and their hybrids generally decreased after peak values at 12 months of age, apparently because of intercurrent diseases (11). Despite the reduction in mean weight, comparable genetic data (Fig. 5) concerning obesity were found at 16 and at 12 months of age (3). Unlike the findings in plasma lipids, evidence for dominance of greater body weight or heterosis in S mice was provided both by analysis of variance and by the disposition of the mean values in the hybrids above the theoretical line of additive genetic behavior (Fig. 5). Although the plasma lipid concentrations and body weights were greater in hybrid and S mice compared to the two lean groups of mice (A and D), there was no simple relationship between obesity and the lipid levels.

Hepatomas. Mice that were heavier at 12 months of age had a greater tendency (significant in 5 of 17 mating groups tested) to have hepatomas when 16 months old. This was particularly true in the S × D crosses; the greater within-litter variance for the F_2 and BC than the genetically uniform groups suggests that this relationship was genetic in nature.

There also was an apparent relationship between hepatomas and high plasma lipid content, particularly with respect to cholesterol + cholesterol ester and phospholipids.

Miscellaneous. No relationship could be demonstrated between the plasma lipid concentrations and coat color, pulmonary adenoma formation or sacral polymorphism. Strain differences were not found in

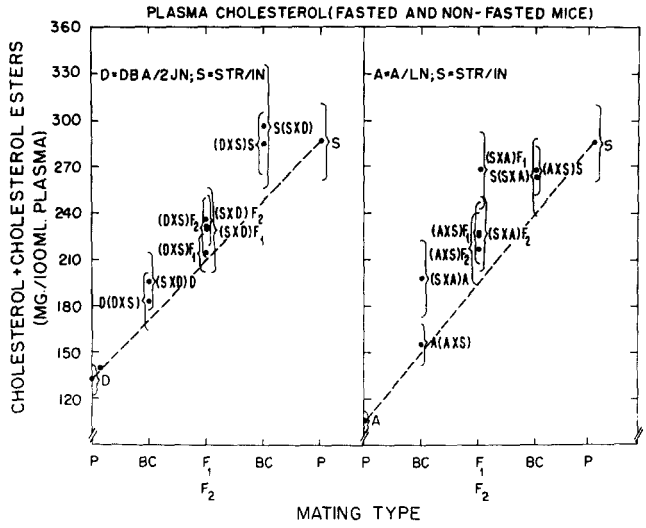

FIG. 3. Plasma cholesterol concentrations (mean ±2 standard errors) in various genetic groups.

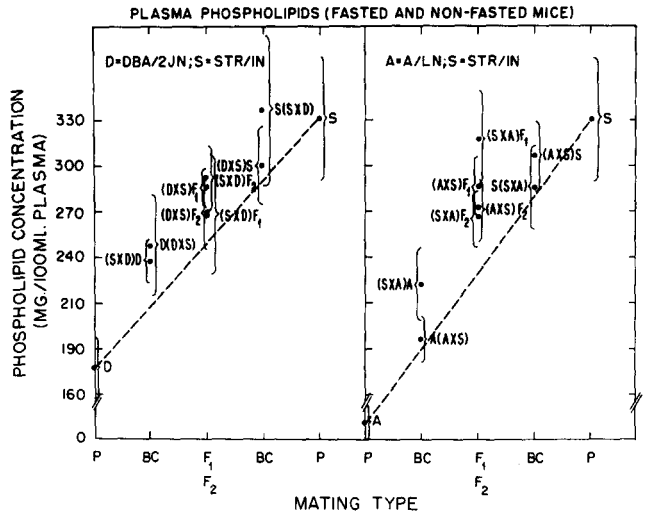

FIG. 4. Plasma phospholipid concentrations (mean ± 2 standard errors) in various genetic groups.

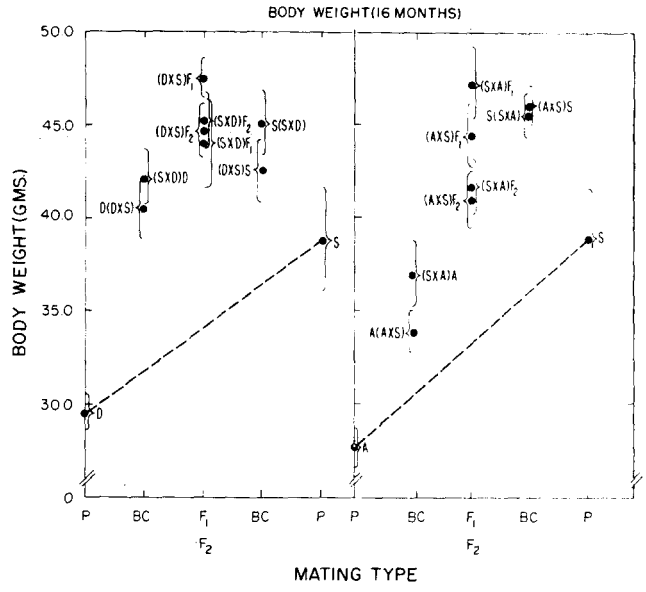

FIG. 5. Mean body weights at 16 months of age in various genetic groups. The brackets enclose two standar errors on either side of the means.

the fasting blood glucose concentrations (S, 87 ± 4.2 (*S.E.*) mg/100 ml; D, 85 ± 4.6; A, 81 ± 7.0). The S mice were relatively anemic throughout their adult life (hemoglobin concentration = 10.5 ± 0.3 (*S.E.*) g/100 ml, hematocrit = 34.4%) unlike the D (12.8 ± 0.1, 41.6 ± 0.2) and A (12.0 ± 0.2, 38.6 ± 0.5) animals. In the P lines, the hematocrits and hemoglobin concentrations were generally inverse to the plasma lipids. In the hybrids, there was no apparent relationship between the plasma lipid values and those for hemoglobin and hematocrit.

DISCUSSION

Although environmental factors, both in the form of fasting and more subtle, unknown influences (disclosed by the between-litter variances), affected the lipid concentrations in the plasma, the genetic nature of the strain differences observed is established by the larger within-litter variances in the F_2 and BC mating groups than in the F_1 hybrids of S and D. The chromosomal rather than maternally acquired origin of the strain differences is demonstrated by the lack of consistent differences in these values in the various reciprocal crosses. The plasma concentrations of total lipids and phospholipids were quite constant throughout the adult life (2–16 months) of the mice. There was a trend for the cholesterol + cholesterol ester values to rise slightly in the 16-month-old groups. Other investigators have found serum cholesterol concentrations in mice to be unaffected by age (2). The absence of sex differences in the plasma lipid content in the present study differs from the findings in other strains reported (2).

The plasma lipid concentrations are the resultant of a number of diverse aspects of lipid metabolism involving intake, synthesis, utilization, and storage. A polygenic character of the inheritance of these concentrations would not be surprising for this reason. The inheritance pattern has a complex character. If a single locus governed the genetic component of the differences among the various groups, the variation observed would have resulted from great differences in environmental sources. In general, the data seem to fit an additive, polygenic interpretation. It is recognized, however, that the variability of the data, the

small numbers involved, and the preliminary nature of the statistical analysis could theoretically serve to cover up segregation of a single gene or dominance. No insight into the mechanisms most important in the genetically determined variations has been gained from the present data. Zomzely and Mayer (1) observed that mice with hyperphagia secondary to hereditary or other metabolic disorders have hypercholesteremia. By contrast, when hyperphagia arises exclusively from damage to brain centers controlling appetite, obese mice have normal cholesterol concentrations. In the present experiment, there was a general correspondence between the variations in the concentrations of each of the fractions studied: cholesterol + cholesterol ester, phospholipids and neutral lipids (as determined by difference from the total lipids) among the various mating groups. The neutral lipid of the plasma, unlike the other fractions, was greatly affected by overnight fasting. A reduction of plasma glycerides of mice following 24-hr fasting has also been reported recently by others (12). In that study, as in the present one, only minor or insignificant changes in phospholipid and cholesterol concentrations occurred as a result of fasting. Although the S mice with high plasma levels are obese, the maintenance of strain differences in cholesterol + cholesterol ester and phospholipid concentrations following fasting indicates that these differences do not have their principal source in the amounts of food ingested. Further evidence for this assumption may be provided by the disparity in the genetic behavior of factors governing body weight and those involved in plasma and hepatic lipid concentrations. The strain differences were not simply the result of over-all changes in hemoconcentration; the lipid values varied independently of the blood glucose and hemoglobin concentrations.

Variations in serum cholesterol concentrations have recently been found by others in strains of mice that were not obese (2). The present findings confirm and extend the dissociation between major genetic factors affecting plasma lipid concentrations and those governing obesity. Although each appears to be polygenic, the over-all behavior with respect to plasma lipids was fairly additive, while greater body weight was dominant. Changing the results of Table 4 to log scale, as suggested by Wright (13) and Chai (14), did not alter these conclusions.

No consistent relationship was found between the plasma and hepatic lipid concentrations, although the lowest values of each occurred in A mice and their hybrids. The low hepatic lipid concentrations of A mice appeared to be related to a maternal factor in this strain: in the various crosses, the hepatic lipid values were lower when the dam was A than in the reciprocal. Whether this maternal factor was of genetic or extrachromosomal type cannot be established from the present data. Since the reciprocal differences carried over into the F_2, in addition to the F_1 and BC mice, an extrachromosomal basis seems more likely. Nevertheless, both high body weight and high plasma levels of cholesterol + cholesterol ester and of phospholipids appeared to be genetically associated with the development of hepatomas. There was no apparent relationship of hepatic lipid concentration to hepatoma formation, but the number of tests performed was too small to be tested statistically.

Genetic variations in the concentrations of plasma lipids have also been noted in other species. In man, hereditary patterns of hyperlipemia and of hypercholesteremia are well documented (15). High and low cholesterol levels have been found in different lines of chickens (16). A strain of spontaneously obese rats with hyperlipemia affecting all three principal fractions, somewhat similar to the values observed in the S mice, has been reported recently (17).

REFERENCES

1. Zomzely, C. and J. Mayer. *Nature* **182**: 1738, 1958.
2. Bruell, J. H., A. F. Daroczy, and H. K. Hellerstein. *Science* **135**: 1071, 1962.
3. Sokoloff, L., L. B. Crittenden, R. S. Yamamoto, and G. E. Jay, Jr. *Arthritis Rheumat.* **5**: 531, 1962.
4. Snell, G. D., J. Staats, M. F. Lyon, L. C. Dunn, H. Grüneberg, P. Hertwig, and W. E. Heston. *Cancer Res.* **20**: 145, 1960.
5. Strumia, M. M., A. B. Sample, and E. D. Hart. *Am. J. Clin. Pathol.* **24**: 1016, 1954.
6. Drabkin, D. L., and J. H. Austin. *J. Biol. Chem.* **112**: 51, 1935.
7. Nelson, N. *J. Biol. Chem.* **153**: 375, 1944; Somogyi, M. *J. Biol. Chem.* **195**: 19, 1952.
8. Bragdon, J. *J. Biol. Chem.* **190**: 513, 1951.
9. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. *Manometric Techniques.* Minneapolis, Burgess Publishing Co., 1957, p. 273.
10. Zak, B., R. C. Dickenman, E. G. White, H. Burnett, and P. J. Cherney. *Am. J. Clin. Pathol.* **24**: 1307, 1954.
11. Sokoloff, L., and M. F. Barile. *Am. J. Pathol.* **41**: 233, 1962.
12. Rehnborg, C. S., J. K. Ashikawa, and A. V. Nichols, *Radiation Res.* **16**: 860, 1962.
13. Wright, S. In *Quantitative Inheritance*, London, Her Majesty's Stationery Office, 1952, pp. 5–41.
14. Chai, C. K. *Genetics* **41**: 165, 1956.
15. Hirschhorn, K., and C. F. Wilkinson, Jr. *Am. J. Med.* **26**: 60, 1959.
16. Hardy, L. B., H. V. Auger, and F. H. Wilcox. *Am. J. Physiol.* **202**: 997, 1962.
17. Zucker, T. F., and L. M. Zucker. *Proc. Soc. Exptl. Biol. Med.* **110**: 165, 1962.