THE INHERITANCE OF THE DIFFERENCE IN THE COMPOSITION OF THE LIVER MITOCHONDRIA BETWEEN TWO MOUSE STRAINS[1,2]

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IT has been conclusively demonstrated that a large number of enzymes are not freely dissolved in the protoplasm but are concentrated on cytoplasmic particles, especially on the mitochondria (rev. LINDBERG and ERNSTER 1954; SCHNEIDER 1953). Because of their great importance in intracellular metabolism, the behavior of the mitochondria in heredity and their variation under the influence of genes becomes a problem of great interest. This problem can be attacked by demonstrating differences in the properties and composition of cytoplasmic particles from the same organ of two different strains of the same species, and by studying their behavior in crosses. It has been shown (CASPARI and SANTWAY 1954) that liver mitochondria isolated from males of the mouse strains C57 Bl and BALB differ in their phosphorus/nitrogen ratios. It could be shown that at least part of this difference is due to an increased amount of ribose nucleic acid, relative to protein, in C57 as compared to BALB. It was furthermore demonstrated that the character "P/N ratio" is a complex character consisting of at least two components: in the mitochondrial fraction sedimented by low centrifugal force C57 males show a higher P/N ratio than BALB males. In the fraction sedimented by higher centrifugal force the P/N ratio appears to be equal in the two strains. But in the latter fraction, a higher proportion of the total mitochondrial material is contained in C57 males than in BALB males. Since the lighter mitochondria are relatively rich in phosphorus, this fact contributes to the higher P/N ratio of the total mitochondrial preparation obtained from C57 males as compared to BALB.

The present paper constitutes an attempt to describe the behavior of this difference in mitochondrial composition in crosses between the two mouse strains C57 Bl and BALB.

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MATERIALS AND METHODS

The highly inbred mouse strains C57 Bl/subline 11, originally obtained from the Roscoe B. Jackson Memorial Laboratory, and BALB/Ci were used. F_1 and F_2 crosses were carried out in both directions. For the purposes of this paper, F_1 hybrids from crosses of C57 ♀ × BALB ♂ will be designated CB, from crosses

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BALB ♀ × C57 ♂ by BC. The CB F_1 was obtained from crosses of 9 C57 females and 2 BALB males, BC F_1 from crosses of 5 BALB females and 2 C57 males. The CB F_2 was derived from 2 CB males and 6 CB females some of which were not sibs. The same applies to BC F_2 for which 3 BC males and 7 BC females were used. In addition, backcross animals from F_1 females from both reciprocal crosses to C57 and BALB males were investigated.

The animals were starved overnight before being used. Liver mitochondria were isolated according to the method of HOGEBOOM, SCHNEIDER and PALLADE (1948), as described previously (CASPARI and SANTWAY 1954). The perfused livers were homogenized in 0.88 M sucrose and, after previous removal of cell debris and nuclei by repeated centrifugation at 600 g, the mitochondria were isolated by centrifugation at 24,000 g for 20 minutes in a Servall SS2 centrifuge. The sediment was washed once in 0.88 M sucrose, suspended in saline and analyzed for nitrogen by the micro-Kjeldahl method and for phosphorus by the Fiske-SubbaRow method. The fluffy sediment described by LAIRD, NYGAARD, RIS and BARTON (1953) was included in the mitochondrial preparations.

It has been shown previously (CASPARI and SANTWAY 1954) that there exists a significant variation in P/N ratios from experiment to experiment. It is therefore of importance to compare animals of different genetic constitutions in the same experiments. In every experiment, eight animals were used since this number could be conveniently handled without involving a long delay between the killing of the animals and the centrifugation. In most experiments, two C57 and two BALB males were used as controls, so that two animals each from two different crosses were compared with each other and with the controls. The ages of the different animals used in the same experiments were approximately equal; no animals under three months of age were used (CASPARI and SANTWAY 1954). In some experiments, four animals each from two different crosses, or two animals each from four different crosses were compared. In some cases, one of the eight samples was lost, either due to accidents during centrifugation, or more frequently because one of the starved animals turned out to be ill or abnormal at dissection. Any animal showing signs of illness or abnormality in any internal organ was excluded from the evaluation. Values obtained in experiments in which one sample was lost, and consequently only one value present for one of the genotypic constitutions, were included in the calculation of means, but were not used in the analyses of variance.

RESULTS

The results obtained from the whole material are summarized in table 1. The table presents the means of the P/N values and their standard errors for each of the strains and crosses. In addition, the table contains an estimate of their variances. As pointed out previously, there exists a significant variability between experiments. Therefore, the values from all experiments involving a particular strain or cross were subjected to an analysis of variance which distinguishes the variance between and within experiments. In all cases, except the two indicated in table 1 by an asterisk, the variance between experiments is significantly, at least at the .05 level, higher than that within experiments. Therefore, in all cases the residual mean square after

TABLE 1

Phosphorus/nitrogen ratios of mitochondria from C57 Bl and BALB males and females and their crosses. In each cross the female parent is designated first; for example, (BC)C means a female from a mating of B female × C male, mated with a C male

Strain	Mean ± SE mg P/gN	*n*	Residual mean square	df
males				
C57 (C)	102.3 ± 0.70	110	19.98	55
BALB (B)	97.7 ± 0.93	109	40.18	54
CB	97.8 ± 1.33	27	14.89	13
BC	98.7 ± 1.48	27	14.50	13
(CB)(CB)	101.8 ± 0.97	53	27.56	28
(BC)(BC)	98.7 ± 1.00	53	38.52	29
(CB)C	101.1 ± 1.35	40	38.82	24
(CB)B	99.6 ± 1.35	24	24.03	12
(BC)C	102.0 ± 0.97	39	18.17	23
(BC)B	102.2 ± 1.74	24	74.15*	12
females				
C57 (C)	100.3 ± 1.30	37	28.73	19
BALB (B)	97.6 ± 1.16	38	15.11	20
CB	92.5 ± 1.59	24	25.61	16
BC	91.1 ± 1.23	23	9.15	15
(CB)(CB)	91.5 ± 0.86	25	9.21	17
(BC)(BC)	90.1 ± 1.20	25	27.12*	17

* Indicates that in these crosses the mean square between experiments is *not* significantly higher than the residual mean square.

elimination of the variance between experiments is taken as an estimate of the true variance, and indicated in table 1.

Males from the pure strains

The data presented in table 1 show a significantly higher mean P/N ratio for C57 males than for BALB males. This confirms the observation of CASPARI and SANTWAY (1954) on a larger material. The average absolute difference, 4.6 mg P/gN is close to the value found previously, 5.7 mg P/gN. In addition, a difference in residual mean squares between the two strains appears from the table; BALB males show a higher variance between individuals than C57 males. This difference is significant at the .02 level ($F = 2.011$, 54 and 55 df, $F_{.02} \sim 1.90$).

F_1 males

Table 1 shows no difference between the reciprocal F_1's. This is borne out by the analysis of variance which gives no indication for a difference between the two crosses (F for strains/individuals = 1.253, df 1 and 38, $F_{.05} = 4.10$). The material consists of 14 experiments, each of which contained two C57 and two BALB males in addition to two males of each reciprocal F_1. The distribution of the values obtained in this way is given in figure 1. It appears that both F_1's resemble strain BALB. This is borne out by the means of the controls for these experiments which are 99.6 ±

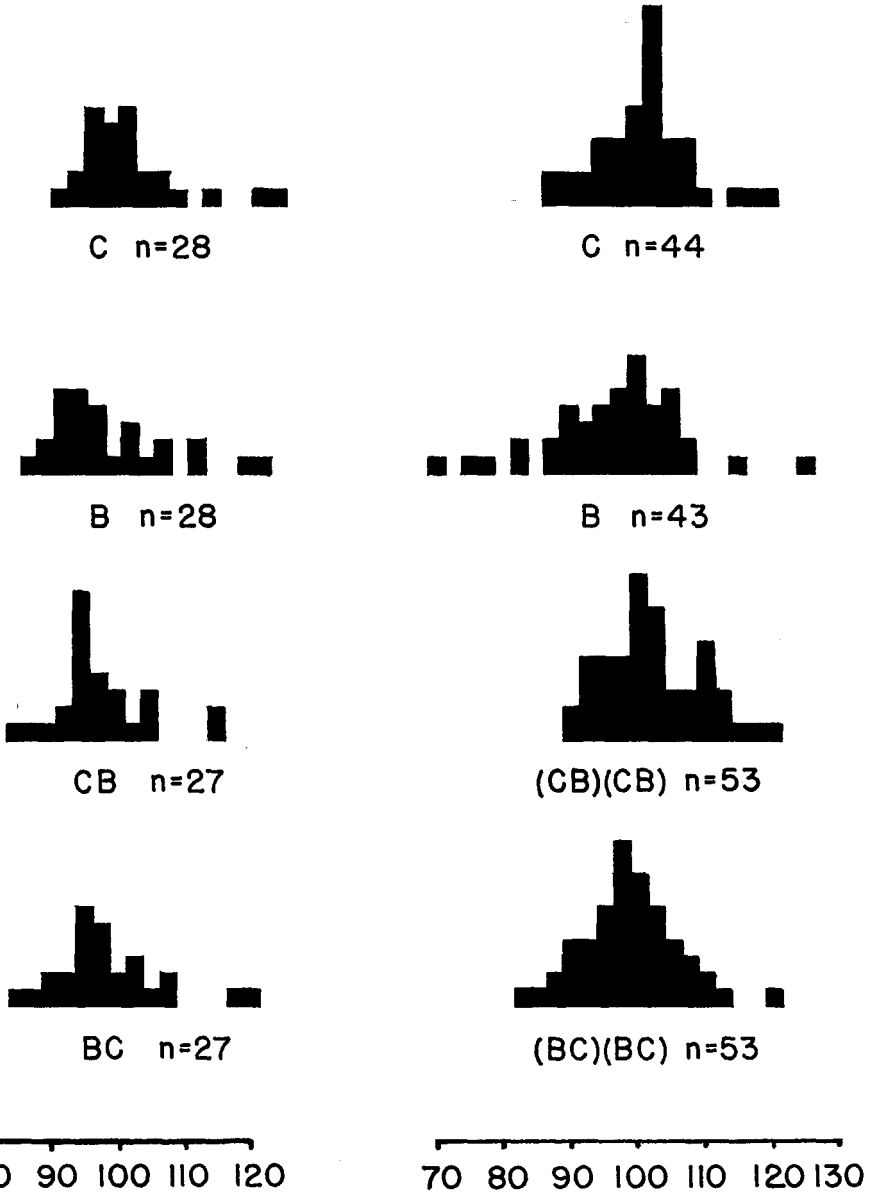

FIGURE 1.—Distribution of values for phosphorus/nitrogen ratios of mitochondria from the livers of F_1 males (left) and F_2 males (right) from reciprocal crosses and their C57 Bl and BALB controls (mg P/gN).

1.72 for BALB and 102.9 ± 1.46 for C57. The difference between the means of both F_1's and C57 is significant at the .05 and .015 level, respectively (t for C57 − BC = 2.014, for C57 − CB = 2.598, for 54 df each).

The residual mean squares of both reciprocal F_1's are lower than those of the in-

TABLE 2

Analysis of variance of phosphorus/nitrogen ratios from reciprocal F_2's

Source of variance	df	Sum of squares	Mean squares
experiments	19	2,374.38	124.97
crosses	1	193.72	193.72
interaction	19	694.35	36.54
individuals	52	1,814.24	34.89
Total	91	5,076.69	

bred strains C57 and BALB. If the two reciprocal F_1's are pooled which is legitimate, because of the lack of a demonstrable difference, an F ratio of 2.728 for 54 and 39 df is obtained for the variances of BALB and the combined F_1's, significant at the .02 level ($F_{.02} \sim 2.08$). The residual mean square of the C57 males is not significantly higher than that of the F_1's.

F_2 males

As shown in table 1, the average P/N ratio of CB F_2 is higher than that of BC F_2. This difference is significant at the 3 percent level ($t = 2.225$, $P < .03$). The same result is obtained by means of analysis of variance, as indicated in table 2. The mean square for the different experiments is, as usual, significantly higher than the residual mean square ($F = 3.538$ for 19 and 71 df, $P < .01$). The variance between strains is significant at the .05 level ($F = 5.484$ for 1 and 71 df, $F_{.05} = 3.98$).

The mean P/N ratios of the control samples for the F_2 are 100.9 ± 1.07 for C57 and 95.6 ± 1.51 for BALB. Comparison with table 1 shows that CB F_2 is very close to C57, and significantly higher than BALB ($t = 3.455$, df = 92, $P < .01$). BC F_2 appears to be intermediate between C57 and BALB; the difference to either one of the control strains is not significant.

The variance of F_2 appears to be increased as compared to F_1. If compared to the combined value for the mean squares of both reciprocal F_1's, CB F_2 has a significantly increased residual variance ($F = 2.615$, 29 and 39 df, $P < .02$). For BC F_2, the difference is in the same direction but not significant ($F = 1.871$, 28 and 39 df, $P < .1$). The distribution of the reciprocal F_2's and their controls is graphically represented in figure 1.

Backcross males

As seen in table 1, no significant difference appears between the means of the backcrosses. In 12 experiments, two males from each of the backcrosses were investigated together. The analysis of variance for these 12 experiments gives no indication for a significant variance due to the different crosses. Further experiments, comparing only (BC ♀ × C57 ♂) with (CB ♀ × C57 ♂) did not give any indication of a difference between these two crosses. In comparison to C57 and BALB in the same experiments, these backcross values appear to be intermediate, but closer to C57. The residual variances, indicated in table 1, are variable. The very high residual mean square observed for males from the cross (BC ♀ × BALB ♂) should be noted.

Influence of color genes

The strains C57 Bl and BALB differ in three major color genes, C57 being *aa BB CC* and BALB *AA bb cc*. In F_2 and the backcrosses, segregation for these genes occurs. An influence of these color genes on the composition of the liver mitochondria would therefore become apparent, if preparations from animals of different color phenotypes isolated in the same experiment are compared. Most of the animals compared were litter mates. The comparison of 27 Agouti and 27 non-Agouti animals showed a difference of 0.548 ± 1.344 mg P/gN in favor of non-Agouti. The average difference for 20 color and albino animals was 0.495 ± 1.886, the average difference for 10 brown and black mice 2.200 ± 4.578. None of these values is significant. There is no evidence that the P/N ratio of the liver mitochondria is affected by the three color genes.

Females

Table 1 shows that the females from strains C57 and BALB differ in their mean P/N ratios in the same direction as the males; but the difference is not significant. However, an analysis of variance for the material from C57 and BALB females, summarized in table 3, indicates a variance due to strains, significant at the .05 level (F = 6.087 for 1 and 54 df, $F_{.05}$ = 4.02).

There are 12 experiments in which two females from each strain are compared with two males from each strain. The mean P/N ratios of the males and females are not significantly different in either strain. The analysis of variance for these experiments is shown in table 4. There is no indication for a significant interaction, except that the interaction strains × sexes × experiments is close to the .05 level of significance (F = 1.92 for 11 and 48 df, $F_{.05}$ = 1.99). There is a highly significant variance due to strains which is expected since the males are known to be significantly different, and the females differ in the same direction (F = 8.20 for 1 and 71 df, $F_{.01}$ = 7.01). The mean square due to sexes is significant at the .05 level (F = 5.64 for 1 and 71 df, $F_{.05}$ = 3.98).

As shown in table 1, there is no significant difference between the means of the reciprocal crosses in F_1 females. Furthermore, the analysis of variance of the reciprocal F_1's gives no indication for a significant mean square due to the two reciprocal crosses. In six out of eight experiments, the F_1 females were compared with C57 females. The values obtained in these experiments are generally low. (The C57 females used in comparisons with F_1 and F_2 are not included in the mean for C57 females reported

TABLE 3

Analysis of variance for phosphorus/nitrogen ratios from C57 and BALB females

Source of variance	df	Sum of squares	Mean squares
experiments	16	2,762.43	172.65
strains	1	133.66	133.66
interaction	16	337.39	21.09
individuals	38	848.22	22.32
Total	71	4,081.70	

TABLE 4

Analysis of variance for phosphorus/nitrogen ratios from C57 and BALB males and females

Source of variance	df	Sum of squares	Mean squares
experiments	11	1,223.09	111.10
strains	1	190.13	190.13
sexes	1	130.90	130.90
interaction strains/sexes	1	0.71	0.71
interaction strains/expts.	11	133.81	12.16
interaction sexes/expts.	11	281.66	25.61
interaction strains/sexes/expts.	11	541.51	49.23
individuals	48	1,230.33	25.63
Total	95	3,732.14	

in table 1.) The experiments show that the means for both F_1's are slightly lower than those of C57 females (means: BC F_1 = 91.0 ± 1.85, CB F_1 = 93.7 ± 2.13, C57 = 96.6 ± 2.06). This difference is significant at the .05 level for BC (t = 2.02, df = 27, $P \sim .05$), but not for CB (t = 0.97, df = 28). Similarly, in F_2 no evidence for a difference between the two reciprocal crosses could be found either by comparison of the means or by analysis of variance. Six experiments in which F_2 females were compared with C57 females indicate that the F_2 means are lower than those of the C57 females (means BC F_2 = 90.3 ± 1.73, CB F_2 = 89.7 ± 0.89, C57 = 95.4 ± 2.28). This difference is significant at the .05 level for CB F_2 (t = 2.32, df = 28, $P < .05$), not significant for BC F_2 (t = 1.78, df = 28, $P \sim .07$).

EVALUATION OF RESULTS

The character investigated, P/N ratio of the mitochondria, is a quantitative character. Since it constitutes a ratio of two values, the possibility exists that it may not be normally distributed. The distributions shown in figure 1 and in figure 1 of CASPARI and SANTWAY (1954) appear, however, sufficiently in agreement with a normal distribution to make the statistical treatment legitimate.

There is no doubt that a significant difference exists between the means of the males of the two strains C57 and BALB. The difference is, however, small, and much overlapping between the two strains occurs. In both strains, there is a certain amount of variability inside experiments, and the variance is significantly higher in BALB than in C57. It cannot be decided to what extent the variability inside the strains is due to genetic heterogeneity, since correlations between parents and offspring have not been established because of the significant variance between experiments. In spite of these difficulties, some facts concerning the crosses are well established. The F_1 males have a P/N ratio similar to BALB and significantly lower than C57. There is no evidence for a difference between the males from reciprocal crosses. In the F_2 males, the difference between the reciprocal crosses is significant at the .03 level and is in the direction of the grandmaternal strains. There appears to be no difference between the males obtained from backcrosses of F_1 females from the two reciprocal crosses to males from the pure strains. All four backcrosses have P/N ratios which appear to approach the C57 controls.

The similarity of F_1 males to BALB may be interpreted to mean that heterozygotes for the genes determining P/N ratio of the mitochondria show the BALB phenotype, independently of the X chromosome, of the cytoplasm and of the Y chromosome. The difference between males from reciprocal crosses found in F_2 must be due either to the cytoplasm or to the Y chromosome, since the reciprocal F_1's must be assumed to be identical with respect to their autosomes and their X chromosomes. Since this difference between reciprocal crosses is not found in F_1, it must be concluded that its appearance in F_2 is due to the establishment of homozygotes. In other words, the high P/N ratio in CB F_2 appears to be due to a cooperation between genes in homozygous condition and the cytoplasm or the Y chromosome.

Whether the factor collaborating with the genes is the cytoplasm or the Y chromosome cannot be decided with certainty. It seems preferable to regard the cytoplasm as responsible since CB F_2 has the Y chromosome from BALB, and it would have to be concluded that the action of the Y chromosomes, as seen in F_2, is opposite to the direction of the difference between the pure strains. In addition, similar cases of interaction of plasmon-sensitive genes with certain cytoplasms have been frequently described in the literature (CASPARI 1948). Dependence on single recessive genes which are expressed only in a specific cytoplasm has been described for pollen-sterility in Linum (BATESON and GAIRDNER 1921), for a flower abnormality in Epilobium (Michaelis 1940), for sex determination in Streptocarpus (OEHLKERS 1941). In analogy to these cases, the genetic behavior of the mitochondria may be interpreted as being influenced by genes which in homozygous condition increase the P/N ratio in C57 cytoplasm but not in BALB cytoplasm. The heterozygotes are not plasmon-sensitive. Segregation of genes affecting mitochondrial P/N ratio is suggested by the fact that both in F_2 and in the backcrosses the variances inside experments are increased as compared to F_1.

The mean P/N ratio of CB F_2 males is indistinguishable from C57. This is astonishing since in F_2 many genes should be present in heterozygous condition, and induce a P/N ratio similar to BALB. As an interpretation, it may be suggested that plasmon-sensitive genes derived from BALB may also induce an increased P/N ratio in C57 cytoplasm when present in homozygous condition. This assumption seems to agree with the finding of MICHAELIS (1942) that genes derived both from the maternal and from the paternal side may appear plasmon-sensitive in crosses, and that in addition their plasmon sensitivity depends on the remainder of the genotype. The assumption that genes derived from BALB in homozygous condition may induce a higher P/N ratio in C57 cytoplasm may also explain the high value obtained in the backcross CB ♀ × BALB ♂. The mean from the backcross BC ♀ × BALB ♂ may be regarded as unreliable because of its high standard error. The high variance inside experiments in this cross recalls the original BALB.

Since the character investigated is a quantitative character, polygenic inheritance may be expected to occur. This is also suggested by the previous finding that the character "P/N ratio" is actually a composite character in which at least two different components are involved. It is, however, impossible on the basis of the data presented to estimate the number of genes involved.

The P/N ratios of the mitochondria from the females of the two strains differ from each other in the same direction as the males. But the difference is quantitatively

smaller and statistically insignificant. Evidence for a genetic basis of the mitochondrial constitution in the females is derived from the fact that both F_1 and F_2 females have lower P/N ratios than C57 females. Of the four comparisons possible, two are significant at the 5 percent level and one more at the 7 percent level; it may be concluded that at least some of these differences are real. The lack of a difference between the pure strains and between the reciprocal F_2's in the females could suggest that the difference in the F_2 males may be due to an influence of the Y chromosomes; but this assumption appears to be unlikely in view of the fact that Y chromosomes would be assumed to act in an opposite direction in the pure strains and in F_2. It appears preferable to assume that the difference in mitochondrial composition between males and females is sex-controlled. This interpretation agrees with the evidence that mitochondria may be affected by hormones. Hexokinase activity, e.g., is known to be influenced by a variety of hormones, including estrogen, and has been shown to be largely restricted to the mitochondrial fraction (CRANE and SOLS 1953).

DISCUSSION

On the basis of the data reported, the mitochondrial P/N ratio appears to be dependent on segregating genes in addition to a cytoplasmic component. This interpretation is in agreement with the genetic behavior of mitochondria in yeast, as analyzed by EPHRUSSI and collaborators (rev. EPHRUSSI 1953). In yeast, differences in mitochondrial activity have been shown to depend on both nuclear genes and cytoplasmic factors.

The manner of interaction between the genes and the cytoplasmic component involved recalls the interaction of genes and plastids in Oenothera, as described by RENNER (1936) and SCHWEMMLE *et al.* (1938). In these cases, a certain plastid constitution is transmitted independently of the genes. This differential plastid constitution is not expressed directly in a phenotypic difference, but in the differential reaction of the chloroplasts to certain genotypic constitutions.

The question arises whether the cytoplasmic factors responsible for the reaction of the mitochondria on chromosomal genes are the maternally transmitted mitochondria themselves. The question whether or not the mitochondria constitute self-reproducing entities has been frequently discussed. The fact that cells without microscopically visible mitochondria have been found to be viable and able to produce visible mitochondria does not exclude the possibility that microscopically invisible stages of mitochondria exist, as has indeed been suggested on the basis of electron-microscopic observations (EICHENBERGER 1953). It has been pointed out by SONNEborn (1950) that the only valid criterion of self-reproduction consists in the occurrence of independent mutations and the transmission of the mutated state to successive generations independently of genotype and environment. This criterion has been met for mitochondria in EPHRUSSI's experiments in yeast and for the mitochondrial elements in a melanotic mouse tumor (WOODS, DUBUY, BURK and HESSELBACH 1949). The observations reported in the present paper point in the same direction. However, caution is indicated because the independent transmission of the difference has been demonstrated for only two generations, and the possibility that the independently transmitted factor may be the Y chromosome is not excluded.

It appears from the data that the variance of mitochondrial P/N ratio in F_1 is

reduced as compared to at least one of the parental strains. A decrease in variance in F_1 has been repeatedly found in experiments involving quantitative characters, e.g., by GRÜNEBERG (1950) in the mouse, by ROBERTSON and REEVE (1952) and by DOBZHANSKY and WALLACE (1953) in Drosophila, and by MATHER (1950) in Primula. This phenomenon is an expression of genetic homeostasis (LERNER 1954; DOBZHANSKY and WALLACE 1953) and is regarded as one of the main factors responsible for the superiority of heterozygotes. It is interpreted to mean that in heterozygous organisms the developmental processes are better buffered than in homozygotes (LERNER 1954; GRÜNEBERG 1954). Superior homeostasis in the heterozygotes with respect to mitochondrial constitution would supply a conceivable physiological mechanism for this phenomenon because of the important function of the mitochondria in cellular metabolism. It has been suggested by LINDBERG and ERNSTER (1954) that one of the main functions of the mitochondria consists in controlling the rate of oxidative breakdown and consequently the availability of substrates for synthetic processes within the cell. Under this assumption, the mitochondria themselves would be important mechanisms in maintaining the homeostasis at the intracellular level. Genetic control of the homeostasis of mitochondrial constitution and function could well represent the physiological basis for the superiority of certain genotypes.

SUMMARY

1. The P/N ratios of isolated liver mitochondria from the mouse strains C57 Bl and BALB and their crosses have been determined.

2. It is confirmed that C57 Bl males show a significantly higher P/N ratio than BALB males.

3. F_1 males have a mitochondrial P/N ratio similar to BALB and significantly lower than C57. No reciprocal differences are found in the F_1 males.

4. The mitochondrial P/N ratios of F_2 males show a difference between reciprocal crosses significant at the 3 percent level. The variance in F_2 is increased over F_1, indicating genic segregation.

5. Males from backcrosses of F_1 females from both reciprocal crosses to males from the original strains do not show any demonstrable differences in P/N ratio.

6. The data are interpreted in terms of interaction of a cytoplasmic factor with plasmon-sensitive genes. A high mitochondrial P/N ratio results from the presence of genes in homozygous condition in the cytoplasm derived from C57. The possibility that the Y chromosome is involved in the determination of the mitochondrial P/N ratio is not excluded.

7. No evidence for an effect of the color genes agouti, black and albino on mitochondrial constitution has been observed.

8. C57 and BALB females differ from each other in the same direction as the males, but not significantly so. Evidence for genetic control of mitochondrial P/N ratio in females is obtained from the fact that in F_1 and F_2 consistently lower values are found than in C57 control females.

9. The variance between individuals is significantly larger in BALB than in C57. The variance in F_1 appears to be reduced. It is suggested that this finding may indicate an increased homeostasis of mitochondrial constitution in the heterozygote,

and that this phenomenon may provide a physiological basis for heterozygote superiority.

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