Proc. Nat. Acad. Sci. USA
Vol. 72, No. 7, pp. 2644–2648, July 1975
Biochemistry

Association of mouse liver adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels with *Histocompatibility-2* genotype

(cell surface/polypeptide hormones/H-2 antigens)

DANIEL MERUELO* AND MICHAEL EDIDIN†

The Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

Communicated by Saul Roseman, May 6, 1975

ABSTRACT **When the content of cyclic AMP (cAMP) was compared in livers of a series of congenic mouse strains differing at the *H-2* locus, significant variation in concentration of cAMP per unit wet weight was found among strains, and also for animals of a given strain with increasing age. For a given age, from 8 to 22 weeks, cAMP levels in liver of $H\text{-}2^a$ and $H\text{-}2^b$ genotype animals were significantly higher than that in liver of $H\text{-}2^k$ type animals. This difference was seen whether the *H-2* gene was on the genetic background of strain C57BL/10, C3H, or A. Levels of cAMP in livers of $H\text{-}2^d$ animals were between those of $H\text{-}2^a$ and $H\text{-}2^k$ animals.**

The *Histocompatibility-2* (*H-2*) gene complex contains the major histocompatibility system of the mouse (1, 2). In addition to genes determining transplantation antigens, the genetic control of several physiological processes, especially immune responses, has been linked with this complex (2, 3). *H-2* linked genes determining the level of secondary immune response to synthetic polypeptides, and the degree of response to low doses of native antigen and to alloantigens have been described. Genetically controlled response to infection with several leukemia viruses is also linked to the *H-2* region (4–8). The linked traits include susceptibility to primary infection and severity of an established infection; they may not be due to immune response against the viruses. Still more general physiological traits appear to be affected by *H-2* region genes. Steroid-sensitive organ weights and the level of plasma complement and steroids all appear to depend to some extent on *H-2* genotype, and characteristic values for these systems segregate with *H-2* in F_2 and backcross animals (9–11).

Some progress has been made in mapping genes responsible for *H-2*-associated physiological effects; at least three of them lie within the *H-2* complex. Studies with recombinant *H-2* mice have shown that the *Ir-1* gene controlling response to synthetic antigens and the genes controlling the immune response to allogeneic myeloma proteins, *Ir-IgA* and *Ir-IgG*, can be separately localized within *H-2*, between *H-2K* and *Ss-Slp* regions (12, 13). The region carrying these three *Ir* genes most probably contains an even larger number of distinct *Ir* genes (13). The factor affecting resistance to Gross virus leukemogenesis has been located in the *K*-end of the *H-2* complex, but it has not been further separated from known genes of the region.

The immune response genes have been discussed in terms of a system of many linked *Ir* genes, the products of which serve as antigen receptors on T cells (2). However, considering all *H-2*-linked genes, including classical transplantation antigens, and the more recently described *Ia* antigens (14), it appears that a common property of *H-2* complex genes is their effect on the cell plasma membrane. All of the antigens determined by the complex, including Ss protein, appear in the surface membranes of some cells (15), while all of the other genes of the complex appear to affect receptor–ligand associations at the cell surface (although in the case of *Hom-1*, the gene affecting plasma steroid levels, the effect may be one step removed from the phenotype measured).

We might expect that still other processes involving interactions at the cell surface are affected by *H-2* genotype, either because many genes affecting membrane phenotypes lie within the complex, or because a relatively small number of genes exert a wide range of effects on membrane function. The present paper demonstrates an association of *H-2* genotype with levels of liver cyclic AMP, a mediator of the action of many polypeptide hormones (16, 17). The data suggest that *H-2*-associated genes control levels of cAMP metabolic enzymes, or else exert some general modifying effect on hormone binding to cells.

MATERIALS AND METHODS

Cyclic [^{3}H]AMP (24–30 Ci/mmol) and [8-^{14}C]ATP (40–50 Ci/mol) were obtained from New England Nuclear Corp. Unlabeled ATP and cAMP, Tris (Sigma 7-9), 3′:5′-cyclic nucleotide phosphodiesterase (beef heart, 0.388 unit/mg of protein, contaminated with 0.00142 unit of 5′-nucleotidase per mg of protein) were obtained from Sigma Chemical Co. AG-1-X2 resin was obtained from Bio-Rad Laboratories. Bovine serum albumin, Fraction V, was obtained from Miles Laboratories.

Inbred mice of strains C57BL/10SgSn, B10.A, B10.BR, B10.D2 (new), A/J, AKR, C3H/DiSn, C3H/HeJ, and C3H.SW were obtained from the Jackson Laboratory, Bar Harbor, Me. They were held in our animal house under a constant lighting schedule for periods of 3 days to 15 weeks before use.

Animals of C57BL/10SgSn ($H\text{-}2^b$), B10.A ($H\text{-}2^a$), B10.BR ($H\text{-}2^k$), and B10.D2 ($H\text{-}2^d$) strains differ from each other only at the *H-2* locus of the IXth linkage group (18), chromosome 17 (19), having otherwise the common genetic background of strain C57BL/10SgSn. Similarly, C3H/DiSn ($H\text{-}2^k$) C3H/HeJ ($H\text{-}2^k$) and C3H.SW ($H\text{-}2^b$) have a C3H genetic background in common; C3H.SW differs from the other two in *H-2*. Sets of strains, whose members reject grafts from other members of the set due to single histocompatibility gene differences are said to be congenic resistant.

Crosses of congenic resistant strains (B10 × B10.BR),

* Present address: Department of Medicine, Division of Immunology, Stanford University School of Medicine, Stanford, Calif. 94305.

† To whom reprint requests should be addressed.

(B10.A × B10.BR) and (B10.D2 × B10.BR) were bred in our animal house to produce first generation (F_1) and second generation (F_2) mice. The latter animals were *H-2* typed as b/b, b/k, k/k, d/d, or d/k by cytotoxicity with specific anti-$H\text{-}2^b$, anti-$H\text{-}2^k$, and anti-$H\text{-}2^d$ sera (20).

Assay of cAMP. cAMP was measured by the method of Gilman (21) using a cAMP-binding protein extracted from bovine skeletal muscle. In this assay a known amount of radiolabeled cAMP is displaced from the binding protein by unlabeled cyclic nucleotide in a tissue extract. Assay data obtained as displacement of radioactivity from the binding protein are converted to absolute values of cAMP per sample by comparing displacement obtained with standard cAMP solutions.

A single preparation of binding protein eluted from DEAE-cellulose was used for all experiments reported here. Its behavior in a standard assay was unchanged over 3 years insofar as samples used during this time gave identical slopes in our assay system, and remained sensitive to the same levels of standard cyclic AMP, 2.5 nM to 0.1 μM. This range of sensitivity was achieved by addition of a protein kinase inhibitor and bovine serum albumin to the assay system (22, 24). These proteins stabilize cAMP-binding protein complexes, and hence extend the sensitivity of the assay to the level indicated above (21, 23). The assay was not linear below 2.5 nM cAMP, even in the presence of inhibitor; this accords well with the reported binding constant for the protein and cAMP, 2.8×10^9 liters/M (24).

Extraction of Liver cAMP. Animals were killed by cervical dislocation. Their livers were excised in less than 30 sec, and frozen in liquid nitrogen; livers were stored in a −70° freezer for 1–4 days before processing. Each frozen liver was weighed, taken to a cold room, and homogenized with a Waring Blendor in 9 ml of cold trichloroacetic acid or perchloric acid. After removal of precipitated protein by centrifugation trichloroacetic acid was extracted with ether, while perchloric acid was neutralized with KOH. The potassium perchlorate that remained soluble after centrifugation in the second procedure did not interfere with further steps in the extraction.

Liver extracts were next taken to dryness by blowing air over them while they were immersed in a 45° water bath. After drying, the extracts were reconstituted in 5 ml of distilled water. To each were added 0.5 ml of 0.3 N $ZnSO_4$ and 0.5 ml of 0.3 N $Ba(OH)_2$. The mixture was chilled in ice for 15 min, after which the precipitate was centrifuged down (1000 × *g* for 10 min) and discarded. This step was found, as has been reported, to remove interfering nucleosides and nucleotides (25).

A tracer amount of [^{3}H]cAMP was added during homogenization to allow computation of recoveries after extraction and purification. The radiochemical purity of this marker was estimated before and after the procedure by electrophoresis in 1% sodium borate buffer, followed by chromatography in 95% ethanol–ammonium acetate (70:30) (26). Recovery of cAMP estimated by counting a portion of the extract averaged 60%, with values for individual extractions forming a normal distribution around this mean.

After the above steps, extracts contained the equivalent of 200–400 mg of tissue wet weight per ml. They were then assayed at five or more dilutions using a minimum of two samples after each dilution. To measure variation in binding during the assay, dilutions of a standard solution of cAMP were intercalated every 50 samples (five animals) and assayed for cAMP content. Large numbers of extracts were assayed once again, several months later, to ensure reproducibility of results. Altogether the data shown for mouse livers resulted from more than 9000 individual determinations.

Assay Procedure. To 200 μl of 50 mM sodium acetate buffer, pH 4.0, were added 1 pmol of [^{3}H]cAMP (9–10,000 cpm, depending upon the specific activity of the preparation), 50 μl of a mixture containing binding protein (18–25 μg), kinase inhibitor (22 μg), and bovine serum albumin (167 μg), and 10–100 μl of liver extract or cAMP standard solution. The amount of binding protein used was sufficient to bind ⅓ to ½ of the added [^{3}H]cAMP. The mixture was incubated between 3 and 15 hr at 4°; binding was found to be maximal by 3 hr and to remain constant for 24 hr. After incubation, samples were diluted with 1.5 ml of potassium phosphate buffer (20 mM), pH 6.0, and filtered through prewashed Millipore filters (HAWP, 0.45 μm pore size). The filters were then washed with 5 ml of the same buffer, dried at 90°, and immersed in scintillation fluid, and their radioactivity was determined. If known amounts of cAMP were added to aliquots of an extract previously measured in the assay, and the cAMP levels were again determined, the new values approximated well the original value plus the amount of added cAMP. For example, 1.8 pmol of cAMP/10 μl added to an extract containing 1.65 pmol of cAMP/10 μl gave 3.3 pmol of cAMP/10 μl when reassayed.

Validation of cAMP Measurements. Treatment of extracts with phosphodiesterase, as described (26), reduced by more than 90% the cAMP detectable in the binding protein assay. Several substances either enhance or diminish the binding of cAMP by skeletal muscle protein. The majority of these interfering compounds were readily precipitated by $Ba(OH)_2$–$ZnSO_4$ treatment without loss of cAMP (25). Under certain conditions, it has been reported that treatment with $Ba(OH)_2$–$ZnSO_4$ converts ATP present in extracts to cAMP (27, 28). This nonenzymatic conversion occurs much faster at room temperature than at 0–4°. For this reason, treatment of extracts was conducted in ice, following, never preceding, the evaporation step described above. No conversion of ATP to cAMP was found using the extraction procedure described above. In one experiment, a 1000-fold excess of [^{14}C]ATP was added together with the [^{3}H]cAMP tracer to each of 10 liver homogenates. cAMP was separated from half of each extract by absorption to and elution from AG-1-X2 ion exchange resin (27), and then dried and treated with $Ba(OH)_2$–$ZnSO_4$ while the other half was treated only with $Ba(OH)_2$–$ZnSO_4$. No difference in cAMP content could

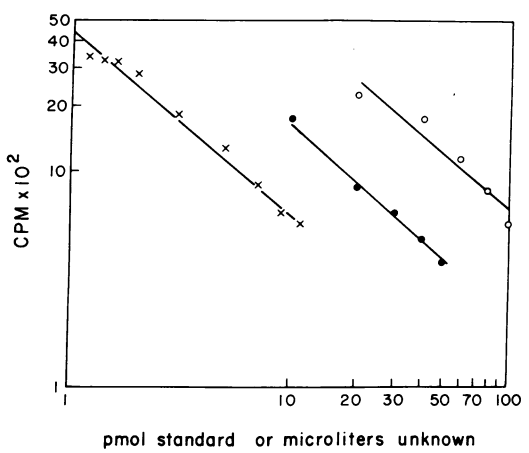

FIG. 1. Assay for cAMP. See *text* for details. Assay mixture contained 0–10 pmol of unlabeled cAMP, ×; 10–50 μl of B10.A tissue extract, ●; or 20–100 μl of B10.BR tissue extract, ○.

Table 1. Levels of liver cAMP in mice of various strains, sexes, and ages

Strain and sex	H-2 type	pmol of cAMP/mg of liver wet weight				
		7–8 Weeks	11–12 Weeks	15 Weeks	17–18 Weeks	19–22 Weeks
B10.A (♂)	a	0.70 ± 0.04	1.52 ± 0.39 1.88 ± 0.25	—	1.98 ± 0.17 1.38 ± 0.28	—
C57BL/10 (♂)	b	0.61 ± 0.04* 0.76 ± 0.03†	1.23 ± 0.10 1.34 ± 0.02 1.20 ± 0.22	1.32 ± 0.32	—	1.34 ± 0.22 1.40 ± 0.30
B10.D2 (♂)	d	0.59 ± 0.02	1.02 ± 0.12	1.42 ± 0.20 1.30 ± 0.38	—	—
B10.BR (♂)	k	0.52 ± 0.02	0.96 ± 0.12 0.80 ± 0.14	—	1.00 ± 0.19 0.88 ± 0.14	0.83 ± 0.13
C3H.SW (♀)	b	1.04 ± 0.10‡	1.30 ± 0.22	—	1.70 ± 0.21	—
C3H/HeJ (♀)	k	0.70 ± 0.03‡	0.97 ± 0.21	—	1.02 ± 0.16	—
A (♂)	a	—	1.48 ± 0.37	1.71 ± 0.20	—	—
AKR (♂)	k	—	0.83 ± 0.24	—	—	—

Each value is the mean of 10 animals and is given ± standard deviation.
* 7 weeks.
† 8½ weeks.
‡ 9½ weeks.

be determined for AG-1-X2-treated and -untreated fractions. Although 5% of the added ATP remained in samples not eluted from a column, this in turn could be absorbed to a fresh AG-1-X2 column.

Statistical Analysis. Fig. 1 shows an assay of standard cAMP and of two liver extracts. In this analytical dilution assay (29) displacement of bound [^{3}H]cAMP by unlabeled cAMP is compared for a standard solution of the nucleotides and for the extracts. Though not shown in the figure, the assay was valid over the range 2.5 nM to 0.1 μM cAMP.

Data obtained from measurements of tissue extracts at several dilutions were fitted to a straight line by a method of least squares. The line obtained was tested for lack of linearity by a goodness of fit test (30). After fitting, covariance analysis (31) was used to test the criterion that no constituent of the sample other than its endogenous cAMP content had an effect on the measurements. When this criterion was met, as is required for analytical dilution assays, the sample and standard lines obtained were parallel to each other. In addition, this analysis was used to determine whether all standards in one assay were equal. If the conditions of parallelism and constant standards were met, then the analysis further indicated differences in liver cAMP content among strains compared. For convenience, data were normalized to liver wet weight, since liver protein/total wet weight did not differ among the four C57BL/10-based congenic strains.

The above analysis tested whether differences existed among a group of strains, but did not indicate which strains differed from each other. For individual comparisons an additional test was required. This test, the Duncan multiple range test, is very much like a Student's t-test for single two-way comparisons, but includes provisions necessary when multiple individual comparisons are made (32). This test was done using a FORTRAN program, BMDO7V, of the UCLA Computing Facility to determine individual strain differences among parental and F_1 and F_2 generations.

RESULTS

Cyclic AMP Levels and H-2. Values of cAMP per mg wet weight of liver are tabulated for animals of the congenic resistant strain series C57BL/10, B10.A, B10.D2, and B10.BR (Table 1). Data are also included for animals of strain C3H/HeH and C3H.SW, another pair of congenic resistant strains, as well as some values for AKR and A/J mice. The data show a clear trend of increase in endogenous cAMP levels with increasing age, in the range of approximately 7–12 weeks of age, with a subsequent plateau to about 18 weeks. Animals older than 22 weeks were not investigated. Preliminary experiments (not shown) gave no indication of sex difference in cAMP for a given strain.

The data points for animals with various *H-2* regions on the C57BL/10 background fell on four distinct curves, three of which are shown in Fig. 2. B10.A mice had the highest levels of liver cAMP and B10.BR the lowest. The lines for B10 and B10.D2 were not statistically significantly different from one another and that for B10.D2 is not shown. The cAMP values determined on 56 animals of the C3H/HeJ–C3H.SW pair, differing from each other at *H-2*, but derived independently of the C57BL/10-based strains, fell on lines for B10.BR and C57BL/10, respectively. Similarly A ($H\text{-}2^a$) mice had higher levels of liver cAMP than did AKR ($H\text{-}2^k$) animals, and the values for the former strains plotted on the B10.A curve while the value for the latter fell on the B10.BR curve. Thus higher levels of liver cAMP were associated with $H\text{-}2^a$ and $H\text{-}2^b$ than with $H\text{-}2^k$ genotypes, regardless of the genetic background on which these alleles were carried. $H\text{-}2^d$ genotype mice had intermediate levels between $H\text{-}2^b$ and $H\text{-}2^k$ mice.

Multiple range tests done separately for each age group bore out the differences indicated when entire curves were compared. As indicated by the curves, significant differences were only detected with animals older than 8 weeks of age.

Breeding Experiments. Reciprocal F_1 hybrids were made between B10, B10.A, B10.D2, and B10.BR animals. Some of these hybrids were used for determination of liver cAMP, while others were mated to give an F_2 generation. Animals of this generation were *H-2* typed and their livers were extracted for cAMP determination. The data for age-matched animals are summarized in Fig. 3 together with the data for

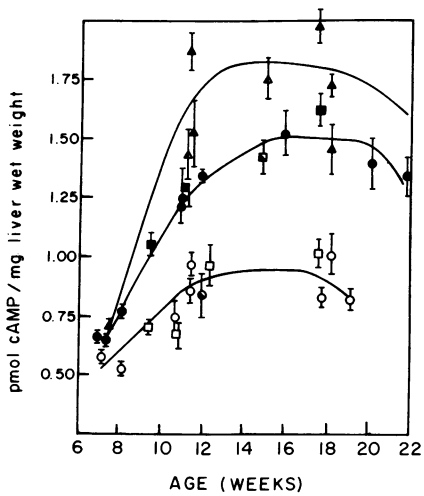

FIG. 2. Effects of age and *H-2* genotype on levels of cAMP per mg of liver wet weight for various strains of mice. Data for B10, ●; C3H.SW, ■; A.BY, ◘; AKR, ◔; B10.BR, ○; and C3H/HeJ mice, □, were plotted as a function of age. Similar data are plotted for B10.A, ▲, and A/J, ▲, mice on a line above that for B10, and C3H.SW. The lines shown are fitted by curvilinear regression technique. Bars indicate standard deviations. Overall, as tested by curvilinear covariance, there were very significant differences among the strains: $F(5,286) = 31.715$ with $F_{0.005}(5, 120) = 3.3499$. The adjusted means for all ages ± the standard error were: C3H/HeJ = 0.75 ± 0.06; B10.BR = 0.78 ± 0.04; B10 = 1.18 ± 0.04; C3H.SW = 1.22 ± 0.07; and B10.A = 1.44 ± 0.05. A multiple range test at the 1% level of confidence indicated no difference in cAMP levels of B10.BR and C3H/HeJ, but showed that these levels were significantly lower than B10.D2, B10, C3H.SW, and B10.A. B10.D2, B10, and C3H.SW mice have, in turn, lower levels of cAMP than B10.A animals. A/J and AKR animals were not included in this analysis because of the limited number of measurements available, but these animals had cAMP levels identical with B10.A and B10.BR, respectively.

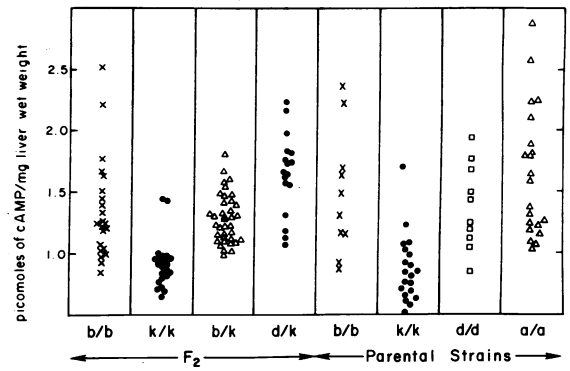

FIG. 3. Segregation of cAMP basal levels in liver with *H-2* in parental strain and F_2 mice. Data shown are for F_2 generations of several independent (B10 × B10.BR), and (B10.D2 × B10.BR) matings. F_2 mice were genotyped (see *Materials and Methods*) as either $H\text{-}2^k/H\text{-}2^k$ (= k/k), $H\text{-}2^b/H\text{-}2^k$ (= b/k), $H\text{-}2^b/H\text{-}2^b$ (= b/b, $H\text{-}2^d/H\text{-}2^k$ (= d/k), or $H\text{-}2^d/H\text{-}2^d$ (= d/d). Values for individual parental strain mice are also shown for comparison. These genotypes are identified in the figure by the abbreviated notation shown in parentheses. The adjusted means ± the standard deviation for the F_2 mice shown were: $(H\text{-}2^k/H\text{-}2^k)F_2 = 0.91 \pm 0.20$; $(H\text{-}2^b/H\text{-}2^k)F_2 = 1.26 \pm 0.18$; $(H\text{-}2^b/H\text{-}2^b)F_2 = 1.32 \pm 0.40$; $(H\text{-}2^d/H\text{-}2^k)F_2 = 1.62 \pm 0.34$. Statistics for parental strains are in the "11–12 week" column of Table 1.

individual parental strain animals; statistical details are given in the legend to this figure. Three points appear from these results. First, cyclic AMP levels of F_2 mice segregate with *H-2* type, and in *H-2* homozygotes their average is well within the range for cAMP in parental strain animals. Second, the cyclic AMP high *H-2* genotypes are dominant over cyclic AMP low genotypes. Thus $H\text{-}2^b/H\text{-}2^k$ animals have levels of cAMP similar to those of b/b homozygote whether in F_1 and F_2 animals [values were determined for (B10/B10.Br)F_1 (b/k) and (B10.D2/B10.Br)F_1 (d/k) and (B10.A/B10.Br)F_1 (a/k) animals as well as for the b/k and d/k F_2 shown in Fig. 4]. Finally, $H\text{-}2^d/H\text{-}2^k$ heterozygotes, whether F_1 or F_2 animals, are anomalous. These offspring of two cAMP low strains have hepatic levels of cAMP as high as those of the $H\text{-}2^a$ strains A/J and B10.A.

DISCUSSION

The use of congenic resistant mouse strains allowed us to state with a fair degree of certainty that the measured differences in basal level of cAMP per mg wet weight among strains were associated with the *H-2* chromosome complex. Furthermore, the associations were not masked by the genetic background, since nearly equal values for liver cAMP were determined for animals with *H-2* regions on three different genetic backgrounds, C57BL/10, C3H, and A.

The reason for increase in cAMP levels with age is not as apparent, though some precedent for this observation is found in observations on changes in enzyme levels with age in rodent liver (33). These changes and the one that we observe do not appear to be due to a change in cellular composition of the liver. Though hepatic parenchyma cells constitute approximately 65% of all liver cells by number, they make up 90% of the liver volume, and presumably about this percentage of tissue weight. The proportion of parenchymal and other cells does not change from birth to several years of age (34).

Tests with F_2 animals from crosses of (B10 × B10.BR), (B10.D2 × B10.BR), and (B10.A × B10.BR) point to a dominant genetic factor or factors affecting cAMP levels in liver identical or closely associated with the *H-2* complex. They also fail to show segregation of any other genetic locus that may have been included when our congenic resistant strains were created. Finally, the crosses rule out environmental effects, since they were all done in a single breeding room.

The fact that B10.A mice, which are suspected ($H\text{-}2^d/H\text{-}2^k$) recombinants, had higher liver cAMP levels than either of the haplotypes from which the recombinant arose, indicates that at least two genes are needed to produce higher levels of liver cAMP. This hypothesis is strongly supported by the finding that the F_1 hybrid between strains with low levels of liver cAMP, B10.D2 and B10.BR, had liver cAMP levels as high as B10.A or A/J animals. Since the suspected crossover for B10.A mice occurred between *Ir-IgG* and *Ss-Slp* (35), it further appears that both ends of the *H-2* region are involved in controlling cAMP levels in liver.

Variation in cAMP levels in liver is thus another quantitative trait apparently linked to the major histocompatibility complex of the mouse. To our knowledge, it is one of the few traits so linked that is clearly involved in more processes than those of the immune system. Liver, though it contains some lymphocytes, is not primarily an organ of immune response. It is, however, a tissue in which numerous hormones converge on a variety of cells, modifying their metabolism *via* the effector cyclic AMP (36). Iványi *et al.* (9, 11) and Hinzova *et al.* (10) demonstrated an association of *H-2* with functions broader than the immune response by showing that *H-2* affected weight of steroid-sensitive organs and levels of complement and plasma steroids. These authors also

suggested that *H-2* region genes had more general effects than on the immune response alone. Both this work and our own raise two questions about the *H-2*-linked genetic control of cell physiology. First, what is the level or point of control and second, what are the implications of this control in whole animals? The answers to the second question are liable to be long in coming, but three mechanisms for genetic control of cAMP levels, which represent cell responses to a wide variety of hormones, come immediately to mind. Two of these would involve differences in activity of adenylate cyclase, the enzyme synthesizing cAMP from ATP, or in 3′:5′-cAMP phosphodiesterase, the cAMP degrading enzyme. Both of these enzymes, although internal to the cell surface, are membrane-associated (37, 39). Differences in their activity would suggest that genes acting on the cytoplasmic side of the cell surface cluster in the *H-2* complex. A third possibility is that the differences in levels of cAMP observed are a direct consequence of the effect of *H-2*-linked genes on the binding of hormones and other molecules to the cell surface. *H-2*-linked genetic control of surface interactions could be exercised in two ways. First, genes determining variant configurations of polypeptide hormone receptors may be present in the *H-2* complex. We would expect that there are at least five of these genes in the region, corresponding to the number of hormones shown to stimulate adenylate cyclase independently (see ref. 38). A second, somewhat less straightforward possibility is that antigenic products of the *H-2* complex modify the interaction of hormones and other ligands with their specific receptors. *H-2* antigens are mobile in the plane of the plasma membrane (40), and probably outnumber hormone receptors in liver and lymphoid cells; their human homologue, HL-A, is present in approximately 50-fold excess over insulin receptor on lymphoid cells (41–43). A receptor surrounded by *H-2* antigens might, because of weak interaction with them, have an altered affinity for hormone.

All of the control points suggested here are at the level of the liver cell's response to hormones. Clearly further points of control might be found, ranging from blood levels of hormones stimulating the liver cells, to the utilization of cAMP by protein kinases, or to its export into the blood. Further experiment on liver cell and homogenate preparations will be required to resolve the possibilities listed here.

We thank Dr. Leonard Herzenberg for suggesting the use of C3H.SW mice, Dr. Alan Gittelson for advice in the statistical analysis of the data, and Mr. Fred G. Bromberg for writing many of our computer programs. Mrs. Maria Eugenia Meruelo and Mr. Steve Gross provided excellent technical skill in the breeding of mice. Mrs. Avril McCheyne provided competence in *H-2* typing of F_2 mice. This work was supported by a National Institutes of Health Grant, AM11202, to M.E. and a training grant to the Department of Biology. This is Contribution no. 832 from the Department of Biology.

1. Démant, P. (1973) *Transplant. Rev.* **15**, 162–200.
2. Shreffler, D. C. & David, C. S. (1975) *Adv. Immunol.* **20**, 125–195.
3. Benacerraf, B. & McDevitt, H. O. (1972) *Science* **175**, 273–279.
4. Iványi, P. (1970) *Curr. Top. Microbiol. Immunol.* **53**, 1–90.
5. Lilly, F. (1971) *Transplant. Proc.* **3**, 1239–1241.
6. Lilly, F. (1968) *J. Exp. Med.* **127**, 465–473.
7. Nandi, S., Haslam, S. & Helmich, C. (1971) *Transplant. Proc.* **3**, 1251–1257.
8. Tennant, J. A. & Snell, G. D. (1968) *J. Nat. Cancer Inst.* **41**, 597–604.
9. Iványi, P., Gregorova, S. & Micková, M. (1972) *Folia Biol.* **18**, 81–97.
10. Hinzova, E., Démant, P. & Iványi, P. (1972) *Folia Biol.* **18**, 237–243.
11. Iványi, P., Hampl, R., Starka, L. & Mickova, M. (1972) *Nature New Biol.* **238**, 280–281.
12. McDevitt, H. O., Deak, B. D., Shreffler, D. C., Klein, J., Stimpfling, J. H. & Snell, G. D. (1972) *J. Exp. Med.* **135**, 1259–1278.
13. Lieberman, R., Paul, W. E., Humphrey, W., Jr. & Stimpfling, J. H. (1972) *J. Exp. Med.* **136**, 1231–1240.
14. David, C. S., Shreffler, D. C. & Frelinger, J. A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2509–2514.
15. Saunders, D. & Edidin, M. (1974) *J. Immunol.* **112**, 2210–2218.
16. Bitensky, M. W. & Gorman, R. E. (1972) *Rec. Progr. Biophys. Mol. Biol.* **26**, 429.
17. Tomasi, V., Trevisani, A. & Barnabei, O. (1971) *Adv. Exp. Med. Biol.* **14**, 245–259.
18. Snell, G. D. & Bunker, H. P. (1965) *Transplantation* **3**, 235–252.
19. Klein, J. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1594–1597.
20. Edidin, M. (1970) *J. Immunol.* **104**, 1303–1306.
21. Gilman, A. G. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 305–312.
22. Appleman, M. M., Birmbaumer, L. & Torres, H. N. (1966) *Arch. Biochem. Biophys.* **116**, 39–43.
23. Walsh, D. A., Perkins, J. P. & Krebs, E. G. (1968) *J. Biol. Chem.* **243**, 3763–3765.
24. Mayer, S. E. (1971) *Ann. N.Y. Acad. Sci.* **185**, 69.
25. Krishna, G., Weiss, B. & Brodie, B. B. (1968) *J. Pharmacol. Exp. Ther.* **163**, 379–385.
26. Meruelo, D., Bromberg, F. G. & Edidin, M. (1975) *Anal. Biochem.*, in press.
27. Brooker, G. (1971) *J. Biol. Chem.* **246**, 7810–7816.
28. Cook, W. H., Lipkin, D. & Markham, R. (1957) *J. Am. Chem. Soc.* **79**, 3607–3608.
29. Finney, D. J. (1952) *Statistical Methods in Biological Assay* (Charles Griffin and Co, Ltd., London).
30. Bliss, C. I. (1967) *Statistics in Biology* (McGraw-Hill Book Co., New York), Vol. I.
31. Dixon, W. J. & Massey, F. J., Jr. (1969) *Introduction to Statistical Analysis* (McGraw Hill, New York), 3rd Ed.
32. Li, J. C. (1964) in *Statistical Inference* (Edwards Brothers, Michigan), Vol. I, p. 270.
33. Wilson, P. D. (1974) *Gerontologia* **18**, 36–54.
34. Fukada, M. & Sibatani, A. (1953) *J. Biochem.* **40**, 95–110.
35. David, C. S., Frelinger, J. A. & Shreffler, D. C. (1974) *Transplantation* **17**, 122–125.
36. Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1968) *Annu. Rev. Biochem.* **37**, 149–174.
37. Davoren, P. R. & Sutherland, E. W. (1963) *J. Biol. Chem.* **238**, 3016–3023.
38. Øye, I. & Sutherland, E. W. (1966) *Biochim. Biophys. Acta* **127**, 347–354.
39. Russell, T. R., Terasaki, W. L. & Appleman, M. M. (1973) *J. Biol. Chem.* **248**, 1334–1340.
40. Frye, L. D. & Edidin, M. (1970) *J. Cell Sci.* **7**, 319–335.
41. Gavin, J. R., Roth, J., Jen, P. & Freychet, P. (1971) *Proc. Nat. Acad. Sci. USA* **69**, 747–751.
42. Sanderson, A. R. & Welsh, K. I. (1974) *Transplantation* **17**, 281–289.
43. Krug, U., Krug, F. & Cuatrecasas, P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2604–2608.