STUDIES OF INTERSPECIFIC (RAT × MOUSE) SOMATIC HYBRIDS. II. LACTATE DEHYDROGENASE AND β-GLUCURONIDASE[1]

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IN the first paper of this series (WEISS and EPHRUSSI 1966), we described the isolation of several different rat × mouse somatic hybrids and some of their properties. Study of their karyotypes showed that, although they lose some chromosomes in the course of successive generations, the hybrid cells retain major portions of both "parental" genomes over prolonged periods of time. The purpose of the experiments described in the present report was to establish whether both sets of chromosomes are functional, i.e., take part in determining the phenotype of the hybrid cells. Although this appeared *a priori* probable (because of the morphology and growth rate of the hybrid cells, generally different from those of either "parent"), more direct evidence seemed desirable and was obtained by the study of two enzymes, lactate dehydrogenase (LDH) and β-glucuronidase, the species origin of which can be determined by their different physical properties. Concerning the first of these enzymes, it has been shown by CAHN, KAPLAN, LEVINE and ZWILLING (1962) that the electrophoretic mobilities of LDH isozymes from rat and mouse organs are clearly different. With respect to β-glucuronidase, PAIGEN's (1961) studies of mutations affecting this enzyme in inbred mouse strains have shown that heat inactivation is an extremely sensitive method for detecting structural differences between enzyme molecules. It was hoped therefore that differences in thermostability of rat and mouse β-glucuronidases would be found which would permit us to distinguish between the enzyme specified by genes of each species in rat × mouse hybrids. It will be seen that this is indeed the case.

Lactate Dehydrogenase

It has been postulated by MARKERT and URSPRUNG (1962) that the active LDH molecule is a tetramer, comprising two types of subunits (sometimes designated as heart and muscle types), specified by nonallelic genes, and which associate randomly in groups of four to produce five different combinations or isozymes. A convincing demonstration of the validity of this hypothesis has been provided by *in vitro* hybridization (MARKERT 1963) and by analysis of mutations affecting one or the other of the two monomer types (NANCE, CLAFLIN and SMITHIES 1963; SHAW and BARTO 1963). More recently, it has been shown by

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SALTHE, CHILSON and KAPLAN (1965) and GOLDBERG (1966) that LDH monomers from different species can also associate to produce active enzyme both *in vivo* and *in vitro*. The present study of rat × mouse somatic hybrids provides a third example of interspecific molecular hybridization *in vivo*.

Tissues and cells: Diaphragm was used as the standard since it has been found, in all species examined, to contain all five LDH isozymes, designated LDH-1 to 5, and separable by electrophoresis (MARKERT 1965). This tissue was obtained from A_2 male rats and from C3H female mice, of three and six months of age, respectively.

The *in vitro* cultured cells used in the persent work have been described in detail (WEISS and EPHRUSSI 1966). They are as follows: Mouse cells—A9 and 1 D; Rat cells—R_{2-3}, R_1 and Recl-C; Hybrid cells—MAT (R_1 × 1 D), DC (Recl-C × 1 D), AC (Recl-C × A9), R_{2-3}D (R_{2-3} × 1 D), R_{2-3}A (R_{2-3} × A9). It will be recalled only that, with the single exception of R_1, a culture derived from whole rat embryo, all cultures used in the present work are clones, isolated either in this or in other laboratories (WEISS and EPHRUSSI 1966).

Preparation of extracts: All cells and tissues were homogenized in an all-glass Potter-Elvehjem homogenizer. Dilutions were made as follows: diaphragm was minced in an equal volume of buffer; cells of "parental" and hybrid lines were homogenized in 0.01 ml of buffer per 50×10^6 and 25×10^6 cells, respectively.

Tris buffer, 0.05 M, pH 7.5, was used for the preparation of all extracts except those employed for *in vitro* hybridization of rat and mouse LDH-5; for the latter operation, phosphate buffer (0.1 M, pH 7.2) was substituted. Homogenates were centrifuged for one hour at 30,900 × *g* in a Servall refrigerated centrifuge, and the supernatant was used as a source of enzyme.

In vitro hybridization was carried out as described by MARKERT (1963): enough NaCl was added to produce a 1 M solution in the phosphate buffered enzyme extract, and the latter was frozen overnight at —20°C.

Electrophoresis and staining: Prior to electrophoresis, cellulose acetate strips (Gelman Manufacturing Company) were soaked for 15 min in the buffer (0.3 M boric acid-NaOH buffer, pH 8.6) described by SMITHIES (1955) for the electrode tray. The strips were blotted with filter paper, and samples of 4 to 6 µl were applied with a Gelman stainless steel wire applicator. The ends of the strips were immersed in buffer, and electrophoresis was carried out at room temperature for one hour, with the voltage set at 200.

The stain used was: 0.025 M Tris buffer, pH 7.4; 0.1 M *d*+*l* lactate, Li salt; 0.005 M KCN; 0.001 M DPN; 0.05 mg/ml of Nitro Blue Tetrazolium; and 20 µg/ml of phenazine methosulfate (ALLEN 1958). This stain was prepared and filtered immediately prior to use. The strips were incubated in this stain for 10 to 20 minutes at room temperature in the dark, fixed for 10 minutes (50% methanol, 10% acetic acid and 40% distilled water), dried and cleared in glycerol.

Results: Figure 1 demonstrates the differences in electrophoretic mobility of lactate dehydrogenase isozymes from rat and mouse. Strips c and d show the patterns obtained with homogenates of rat (c) and mouse (d) diaphragm. It can be seen that while the relative mobilities of LDH-1 (the most rapidly migrating isozymes) from the two species are practically the same, the mobilities of LDH's 2 to 5 are different. The greatest difference is between the most slowly migrating LDH-5 isozymes.

The bands of LDH activity from the two rat "parent" lines, Recl-C and R_{2-3}, are demonstrated on Figure 1, a-R and b-L, respectively. On both strips, extracts of these cell lines have been run adjacent to extract of rat diaphragm; since small variations in the absolute distance of migration from the origin occur from strip to strip, extracts to be compared have been run on the same strip. It can be seen that the only pronounced activity of Recl-C and R_{2-3} clearly corresponds to

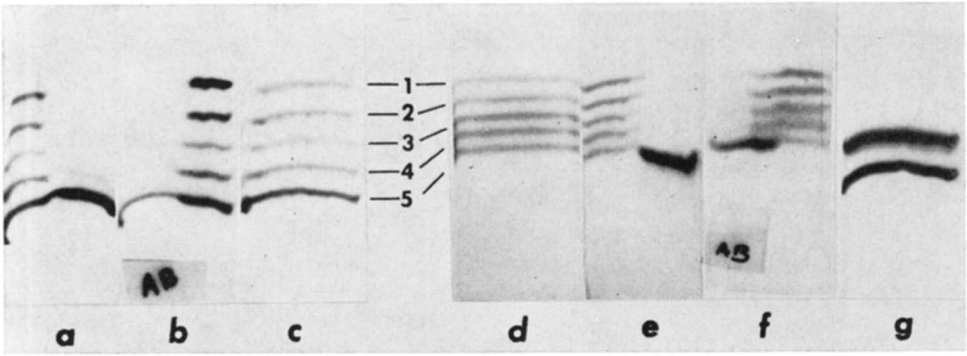

FIGURE 1.—Lactate dehydrogenase patterns, on cellulose acetate strips, of diaphragm and of *in vitro* cultured cells derived from rat and mouse: (a-L) rat diaphragm, (a-R) Recl-C; (b-L) R_{2-3}, (b-R) rat diaphragm; (c) rat diaphragm; (d) mouse diaphragm, (e-L) mouse diaphragm, (e-R) 1 D; (f-L) A9, (f-R) mouse diaphragm, and (g) mixture of Recl-C and 1 D. Each strip is designated by a letter; when two extracts were run on the same strip, the right and left zymograms are referred to as R and L.

LDH-5 of diaphragm. A barely perceptible trace of LDH-4 is observed in some extracts, such as that on strip a-R.

Strips e-R and f-L (Figure 1) show the LDH patterns of extracts from mouse lines 1 D and A9, respectively, run next to extract of mouse diaphragm. The equivalence of the single band of each extract to mouse LDH-5 is obvious.

The last zymogram (g) seen on Figure 1 was obtained by electrophoresis of a mixture of equal quantities of rat (Recl-C) and mouse (1 D) extracts in phosphate buffer. This zymogram demonstrates: (1) the absence of spontaneous hybridization between pure extracts of rat and mouse cells, even when phosphate buffer is used instead of Tris, and (2) the difference in relative mobility of rat and mouse LDH-5 under the described conditions.

The zymograms of Figure 2 show the LDH patterns of several hybrid clones as well as of some of the parents. Thus, strips a, b and c show the isozymes of a

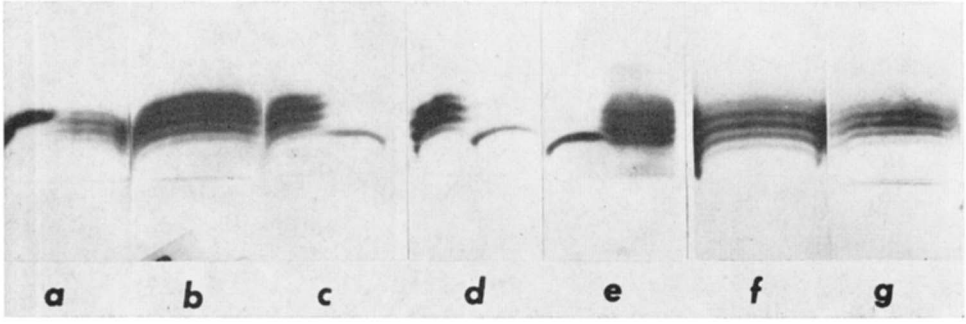

FIGURE 2.—Lactate dehydrogenase patterns of *in vitro* cultured "parent" cells and of somatic hybrids: (a-L) 1 D, (a-R) $R_{2-3}D$; (b) $R_{2-3}D$: (c-L) $R_{2-3}D$, (c-R) R_{2-3}; (d-L) $R_{2-3}A$, (d-R) R_{2-3}; (e-L) Recl-C, (e-R) AC; (f) and (g) MAT-8. For explanation of notations, see Figure 1 legend.

single hybrid clone and of both of its parents. (Strip a-L shows the LDH from the mouse parent, 1 D, and strip a-R that from the hybrid $R_{2-3}D$. Strip b was run with only hybrid extract, and c with hybrid on the left and rat parent on the right.) Examination of these three zymograms permits several conclusions. The hybrid has five LDH bands; the band which migrates most rapidly corresponds to the single LDH-5 band seen in the mouse cells, and the slowest band is equivalent to rat LDH-5. The very pale LDH-4 band of line R_{2-3} corresponds to the second band of the hybrid.

If two types of subunits (one from each parent) are randomly associated into tetramers, all possible combinations would produce all five isozymes, in proportions conforming to the binomial distribution. As expected, five bands of activity were observed in the hybrid cells. Denoting the rat and mouse monomers of LDH as R and M respectively, we can describe the five isozymes formed in the hybrids as RRRR, RRRM, RRMM, RMMM, and MMMM. In some cases however, the bands corresponding to the pure parental isozymes did not stain with equal intensity; in fact, strips b and c of Figure 2 have been overstained to show the very pale fifth band corresponding to rat LDH-5.

Zymograms d through g of Figure 2 contain extracts from other hybrid lines chosen to demonstrate differences observed in the relative proportions of isozymes. Figure 2, d-L, demonstrates LDH from a very young $R_{2-3}A$ hybrid which also shows only a very pale band of pure rat enzyme after approximately 30 cell divisions. Hybrid line AC showed only four bands (e-R) after one month of growth (30 cell generations). Comparison with the zymogram of the rat parent, shown on strip e-L, allows us to conclude that the band missing in AC corresponds to the pure mouse tetramer which, although probably present, does not represent a sufficiently large component to allow resolution by this method.

Strips f and g of Figure 2 show the isozyme pattern of MAT-8, examined after 2 and 8 months *in vitro*, respectively (about 60 and 250 cell generations). While karyological observations of this clone have shown chromosome loss of about 10% between 1 and 8 months *in vitro*, the LDH pattern has remained remarkably constant, with both pure "parental" LDH-5 bands of very similar activity.

It will have been noticed that the LDH pattern of R_1 (the rat parent of MAT) is not shown in Figure 2. This pattern was examined in preliminary experiments, using starch gel electrophoresis. Four bands were observed, corresponding to LDH's 1 to 4. Examined by the same method, the MAT hybrids showed, like all other hybrids, only the five LDH-5 bands.

At first sight, these observations suggest that hybridization resulted in a modification of the pattern of LDH synthesis by the rat genome. It must be kept in mind however, that culture R_1 was obtained from a whole embryo and was composed of cells from many different organs and tissues. Hence, it could not be determined whether the absence in the hybrid cells of rat LDH-1 subunits was due to a change in pattern of synthesis rather than to "mating" of a minority cell type which exhibited primarily LDH-5 activity before "mating".

In an attempt to differentiate between these two possible interpretations, a clone (R_{2-3}) was isolated from a population similar to R_1 in its origin and its LDH pattern. Extracts of this clone exhibited only LDH-5 and a trace of LDH-4 (Figure 1, b-L). Thus, in the case of the MAT hybrids, it is impossible at the present time to eliminate one of the two interpretations suggested above.

Figure 3 a-L demonstrates the LDH pattern obtained by *in vitro* hybridization

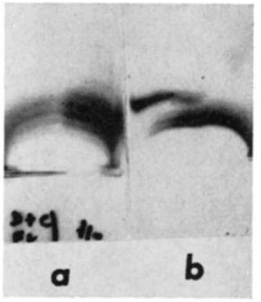

FIGURE 3.—Demonstration of *in vitro* hybridization of lactate dehydrogenase from rat and mouse "parental" cells: (a-L) 1 part Recl-C: 1 part 1 D , (a-R) DC; (b-L) 1 D, (b-R) 6 parts Recl-C: 1 part 1 D. For explanation of notations, see Figure 1 legend.

of extracts of "parent cells", Recl-C and 1 D (one part of extract from Recl-C was added to one part of extract from 1 D, frozen and thawed in 1 M NaCl). It can be clearly seen that the pattern produced is very similar to that of somatic hybrid DC (a-R), obtained by "mating" of these two cell lines. It will be noticed also that neither the pattern of the artificial mixture, nor that of the somatic hybrid conforms exactly to the 1:4:6:4:1 distribution expected on the basis of equal activities of the two enzymes. Instead, it appears that bands 2 and 5 are of approximately equal intensity, and that the same holds for bands 3 and 4. It must be kept in mind however, that the dilutions involved in the preparation of extracts were made on the basis of equality of cell numbers (as would be significant in cell "mating") and not of equality of specific activities.

Figure 3 b, L and R show respectively, zymograms of extract from mouse parent 1 D and of a mixture of rat and mouse extracts which was treated to produce *in vitro* hybridization. The proportions of the parental extracts in the mixture were modified (one part of mouse extract was added to six parts of rat extract) so that fewer than five bands would be observed. In this way, it was possible to test whether known inequalities in proportions of the pure LDH-5's would produce the expected skewed distribution; the pattern produced does not appear to be inconsistent with the expected intensities of the three bands (48.3%, 38.6%, 1.5%).

Conclusions: By demonstrating the presence of hybrid rat-mouse LDH-5 molecules, the described experiments provide a first example of active synthesis of specific proteins by homologous genes of the two parents of an interspecific hybrid. Figure 1 g shows the absence of spontaneous hybridization in mixtures of parental extracts; therefore, examination of LDH pattern in rat × mouse somatic hybrids offers an additional or alternative means of identification of cells as hybrids.

Only LDH-5 of rat and mouse, and hybrid isozymes formed by association of rat and mouse subunits, are seen in the hybrid cells. Since the parental cell populations are characterized by the presence of practically only LDH-5's, this is what was expected on the basis of results of *in vitro* hybridization (cf. MARKERT 1965). (It will be recalled that the rat "parent" cultures contain a trace of LDH-4.

However, the failure to observe LDH-4 bands in the hybrids is not surprising since hybridization with mouse presents additional opportunities for association of subunits.)

Although the closeness of the bands interfered with precise quantitative measurement of their intensities, the proportions of isozymes in all hybrids examined appear to be compatible with the hypothesis of random association of rat and mouse subunits. Some hybrids showed only a very pale band corresponding to one of the pure parental isozymes. This finding may be correlated with the observations of karyotypic changes previously described (WEISS and EPHRUSSI 1966): it may reflect the loss of one of the parental chromosomes carrying an LDH-5 gene. However, no hybrids have been observed which show complete absence of one parental type of LDH-5.

The correspondence of bands obtained by electrophoresis of extracts of somatic hybrids and of hybridization mixtures suggests that the same mechanism of association of monomers operates in the cell and in the test tube. There is no evidence for enzyme mediated association of subunits since *in vitro* hybridization of crystalline LDH yields the same result as a crude extract, i.e., the proportions of isozymes obtained depend only on the proportions of the two monomer types (MARKERT 1963). These observations do not preclude the existence of intracellular micro-environments leading to nonrandom association of monomers, a possibility which has been discussed by VESSELL (1966) in a consideration of the hypothesized relations between LDH-5 and the cell nucleus. However, in the somatic hybrids, there does not appear to be preferential association of subunits of similar species origin.

β-glucuronidase

β-glucuronidase catalyzes the hydrolysis of β-*d*-glucopyranurides, with no apparent specificity for the nature of the aglycone. Although this enzyme is ubiquitous, its physiological function remains unknown. It is found in both lysosomal and microsomal fractions of mammalian tissues; no differences have been detected between the enzyme from these two intracellular sites (PAIGEN 1961).

β-glucuronidase from several species shows two pH optima, at pH 4.6 and 5.2. Although two highly purified preparations of β-glucuronidases have been described (FISHMAN and BERNFELD 1955; LEVVY, MCALLAN and MARSH 1958), no molecular weight determinations have been reported, nor have data been obtained revealing the structure of the enzyme molecule.

The discovery of greatly reduced β-glucuronidase activity in inbred C3H mice led to experiments which showed that it is due to a single gene mutation (LAW, MORROW and GREENSPAN 1952). Further characterization of the mutant and wild-type β-glucuronidases showed that although they are very similar in many respects, they can be distinguished by their very different heat sensitivities (PAIGEN 1961). This technique has also been used with mouse × mouse somatic hybrids to demonstrate that both parental enzymes (C3H mutant and wild type) are present in somatic hybrids (GANSCHOW 1966).

Cells: One line of mouse cells (1 D), derived from a C3H mouse, a strain of rat cells (Recl-C) and two hybrid lines (MAT and DC) were examined.

Preparation of homogenates: The procedure described by PAIGEN (1961) was followed. Cells and tissues were homogenized in the cold in an all-glass Potter- Elvehjem homogenizer, after dilution with distilled water or 0.1 M acetate buffer, pH 4.6. Triton X-100 was added and the homogenate agitated. Before assay, the homogenates were diluted, and the assay performed on the equivalent of 5×10^5, 1×10^6 or 2×10^6 cells per tube.

Partial purification: Each homogenate, prepared as above, was incubated 10 min at 37°C, centrifuged 10 min at $1470 \times g$, and the supernatant recovered. The pellet was washed once with buffer and recentrifuged. The second supernatant was combined with the first one, and centrifuged 30 min at $105{,}000 \times g$ in a Spinco Model L ultracentrifuge. The supernatant was used as the source of enzyme, and showed a 10 to 100-fold increase in specific activity with 100% recovery of total enzyme activity.

Heat inactivation: Appropriate quantities of crude or partially purified enzyme were pipetted into a series of conical glass centrifuge tubes. Acetate buffer, pH 4.6, was added to give a standard ionic strength of 0.1 M acetate in a final volume of 1.5 ml. Duplicate tubes were heated for various intervals, ranging from 0 to 65 minutes at 65°C, and inactivation was arrested by placing the tubes into ice. Substrate solution was added to the cooled tubes, and the standard assay was performed.

Enzyme assay: The substrate solution contained: 0.1 M acetate buffer (pH 4.6), 4×10^{-3} M paranitrophenyl β-*d*-glucuronic acid (pNPG, Cyclo Company, Los Angeles, California), and 0.4% Triton X-100 (cf. GANSCHOW 1966). The volume of each assay tube was 2.0 ml, of which 0.5 ml was substrate solution, and 1.5 ml enzyme plus buffer.

Substrate was added to the cooled tubes. The tubes were then incubated 2 hr at 37°C. The formation of product is linear with time during this period. Following incubation, the tubes were placed in boiling water for one minute. Distilled water (1.0 ml) was added to all tubes which were thereafter centrifuged 10 min at $1470 \times g$.

The supernatant was pipetted into cuvettes (1 cm light path) and read in a Beckman DU spectrophotometer at 415 mμ. After the initial reading (OD_a), the color of released para-nitrophenol (pNP) was developed by adding *ca.* 5 μl of 7 M 2-methyl-2-amino-1:3-propanediol. Final pH in the cuvette was 9.5 to 10.0. A second reading (OD_b) was made at 415 mμ, and the first reading was subtracted from it. Correction for absorbance of the substrate after the addition of alkali was made by running a substrate blank and subtracting the OD change from the value of $OD_b - OD_a$. The resulting optical density was considered as the corrected ΔOD. At 415 mμ, the absorbance of pNP was found to be a linear function of concentration over a wide range. Specific activity units of β-glucuronidase were expressed as μg pNP formed (at pH 4.6 in 2 hours at 37°C) per mg protein. Protein was assayed by the method of LOWRY, ROSEBROUGH, FARR and RANDALL (1951). Bovine serum albumin was used as the standard.

Results: Figure 4 (solid lines) shows heat inactivation curves, plotted as log percent surviving activity, of two rat × mouse hybrid clones, DC-1 and DC-2, and of their "parents," clones 1 D and Recl-C. It can be seen that the heat sensitivities of the enzymes of the hybrids are intermediate between those of the (more resistant) mouse parent and of the (more sensitive) rat parent. These observations appear to warrant the conclusion that genes from both rat and mouse, specifying the structure of β-glucuronidase, are active in the hybrids tested, as evidenced by the presence of enzyme with intermediate heat stability.

The kinetics of heat inactivation, as reflected in the shapes of the heat inactivation curves of the "parental" enzymes, appear to be more complex than would be expected of a population of identical molecules: the inactivation curves of both rat and mouse enzyme appear to be two-slope rather than straight lines (Figure 4). It appears that in both rat and mouse homogenates, two populations of en-

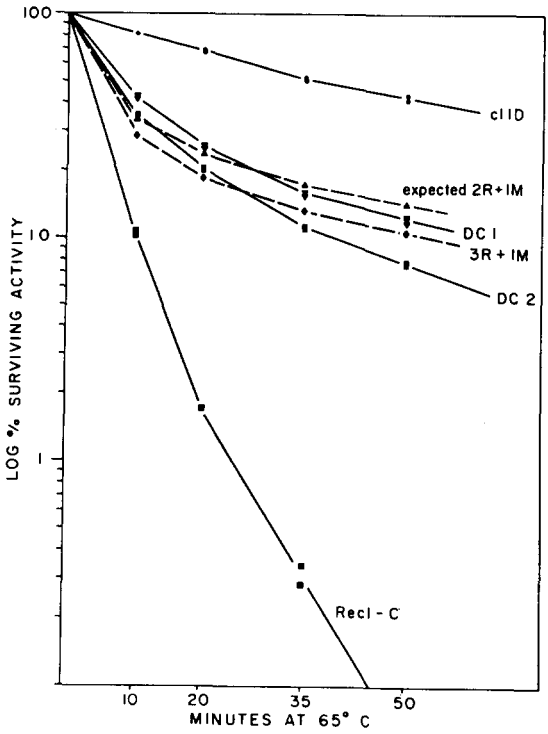

FIGURE 4.—Heat inactivation curves of β-glucuronidase from 1 D, DC-1, DC-2, and Recl-C. The two curves drawn with dashed lines represent expected inactivation of "calculated mixtures," containing 2 and 3 parts of rat activity to 1 of mouse.

zyme molecules are present, one more heat stable than the other. However, since the inactivation experiments were not performed on purified enzyme preparations, this interpretation must be subject to caution. GANSCHOW (1966), who made similar observations, ruled out two possible interpretations: (a) that enzymes from the two intracellular sites, lysosomes and microsomes, may have different inactivation rates, and (b) that the two-slope curves are artifacts of heating. A protection or interference due to the presence of other proteins seems unlikely since crude and partially purified enzyme show the same inactivation kinetics. Finally, no conclusion can be drawn from the heat inactivation curves of the enzymes of the hybrids since their shape suggests, if anything, even more complex kinetics.

The inactivation curves of rat and mouse enzymes enable us however, to calculate the expected inactivation curves of mixtures containing varying proportions of the two "parental" enzymes, and to compare them with the observed inactivation curves of enzymes of hybrid cells. The results of such calculations are given in Figure 4, where the curves of two "calculated mixtures," which most closely fit the curves obtained with hybrid cells, have been drawn in with dashed lines. It can be seen that the inactivation of enzyme of the hybrids is (a) slower than expected during the first 20 minutes, and (b) faster than expected after 30

minutes at 65°C, when rat enzyme no longer survives in significant quantity, and when the curve of the extract from hybrid is expected to show the same slope as that of the mouse enzyme. In other words, the "expected" and observed curves intersect after 20 to 30 minutes at 65°C. A number of such experiments have been performed, using hybrid lines MAT and DC, and in every case the same discrepancy has been observed, suggesting that some interaction (inhibition, activation, protection, etc.) between the mouse and rat enzymes occurs when they are mixed.

In order to test this hypothesis, a 1:3 mixture of extracts of 1 D and Recl-C was prepared and subjected to inactivation at 65°C. The results of this experiment, as well as the "calculated curve" for the same mixture, are shown in Figure 5. Extremely close agreement is seen between the expected and observed result. Thus, the deviation of the inactivation kinetics of the enzyme from hybrid cells from the expected kinetics cannot be ascribed to some trivial interaction between the two types of enzymes in a mixture.

Since the discrepancy between the "expected" and observed inactivation curves of enzyme from hybrid cells was not duplicated by the mixing experiment, it was thought that β-glucuronidase may be a polymer, the monomers of which form, in the somatic hybrids, "interspecific hybrid molecules" with intermediate

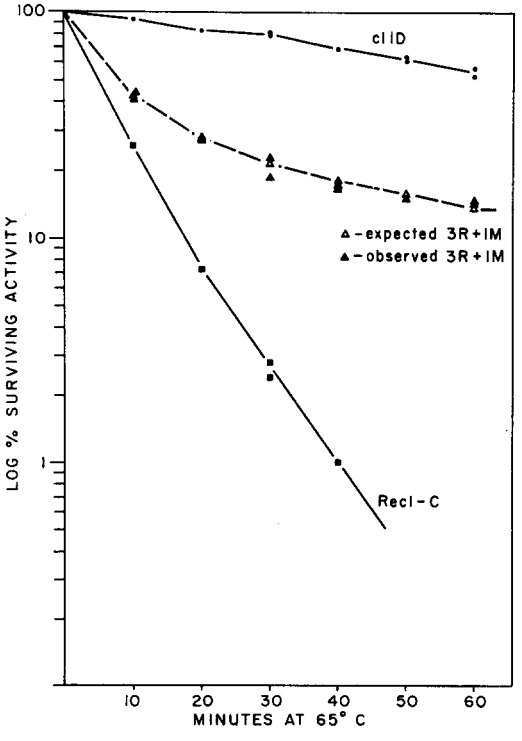

FIGURE 5.—Heat inactivation curves of β-glucuronidase from 1 D, Recl-C, and of an artificial mixture, containing 3 parts of rat activity and 1 of mouse. ▲ experimental values; △ "calculated" or expected values.

properties. This interpretation is compatible with the two observations related above, namely (a) the slower than expected inactivation at the beginning of incubation at 65°C, and (b) the faster than expected inactivation during the latter half of incubation. Preliminary experiments with starch gel electrophoresis (WEISS and EPHRUSSI, unpublished) have shown that β-glucuronidases from the rat and mouse "parental" cells (as demonstrated by incubation with the fluorescent substrate, 4-methylumbelliferone-glucuronide, kindly supplied to us by DR. ROGER GANSCHOW) migrate as single, wide, slightly overlapping bands. Enzyme from hybrid cells also migrates as a single wide band, which fails to reach the outer margins of the single, very wide band observed when a mixture of rat and mouse enzyme is subjected to electrophoresis. These observations, schematized in Figure 6, suggest that hybrid (rat-mouse) enzyme molecules are indeed formed, and that they account for a large proportion of β-glucuronidase activity in hybrid cells.

Conclusions: On the assumption that β-glucuronidase in rat × mouse hybrids behaves similarly to lactate dehydrogenase in the small cells, and to collagen in mouse × mouse somatic hybrids (GREEN, EPHRUSSI, YOSHIDA and HAMERMAN 1966), the specific activity of this enzyme should not differ greatly from that of the average of the specific activities of the "parental" cells. In fact, specific activity measurements of the parental cells reveal that the rat cells possess ten times more activity than the mouse cells (average values in specific activity units: Recl-C, 10.8; 1 D, 1.1), while the various hybrid populations show three to five times more activity than the mouse "parental" cells (DC clones gave values of 4.2 to 5.4). However, although specific activity measurements are in agreement with expectation, calculations of enzyme composition (from heat inactivation rates of artificial mixtures of rat and mouse enzyme) are not; as shown in Figure 4, the "expected mixtures" which most closely approximate the inactivation curves of the hybrid are in porportions of 2:1 or 3:1 (rat activity : mouse activity). Comparison of these values to the composition expected on the basis of specific activities of parental cells, namely *ca.* 10 parts of rat to 1 part of mouse activity, shows that more heat stable (mouse-like) enzyme, and less heat labile

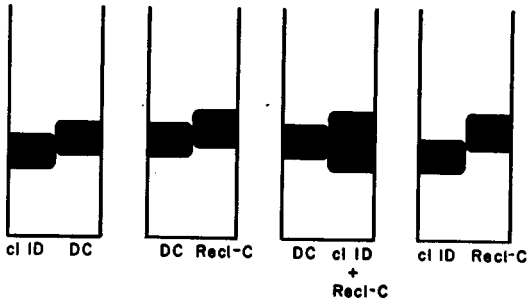

FIGURE 6.—Diagram of β-glucuronidase zymograms. The gels contained extracts from Recl-C, 1 D and DC, as well as a mixture of the parental cell extracts. The electrophoretic mobility of the enzyme from the hybrid cells is clearly intermediate between those from Recl-C and 1 D, and does not correspond to that of the mixture of parental cell extracts.

(rat-like) enzyme is observed than expected. Some possible explanations for this divergence are: (1) a modification of the rates of synthesis of rat and mouse monomers, (2) differences in the rate of their degradation, and (3) nonintermediate activity or heat stability of hybrid molecules.

DISCUSSION

In the first publication of this series, it was shown (1) that the rat × mouse somatic hybrids are capable of indefinite growth, some populations having been propagated for 260 generations, and (2) that the evolution of their karyotypes involves some loss (5 to 10% on the average) of chromosomes, occurring mostly during the first four months of culture. Moreover, after a period of growth, clonal populations become karyotypically heterogeneous, with variants above and below the mean, with respect to marker chromosomes from the two species, as well as to the total number of chromosomes.

The present experiments provide evidence that, in the hybrid cells, the genomes of both parental species are active in the synthesis of the two enzymes examined, lactate dehydrogenase and β-glucuronidase. In both cases, it has been shown that homologous but structurally distinct proteins, from different species, possessing the same enzyme activity, can be distinguished and/or separated.

These facts lead us to believe that studies of interspecific somatic hybrids represent a promising approach to the analysis of gene expression in mammalian cells. The probable presence of a large number of "inbuilt genetic markers," such as LDH and β-glucuronidase, taken together with the occurrence in mass cultures of numerous karyotypic variants, which can be isolated by cloning, should permit (1) the detection of differential effects which manipulations of experimental conditions may have on the appearance of gene products of one or the other species, and (2) a precise correlation of genotype and phenotype.

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SUMMARY

Evidence has been presented which shows that both "parental" genomes are functional in the determination of the phenotype of rat × mouse somatic hybrids. —Isozymes of lactate dehydrogenase (LDH) were examined in parental and hybrid cells. The former were found to be characterized by the presence of LDH-5 alone, rat and mouse LDH's showing different electrophoretic mobilities. The hybrid cells contained LDH-5 from both species, as well as three hybrid rat-mouse LDH-5 isozymes. *In vitro* hybridization of parental extracts produced patterns like those found in the hybrid cells.—β-glucuronidase from parental and hybrid cells was analyzed, using heat inactivation and electrophoresis as means of distinguishing rat and mouse activity. Enzyme with intermediate heat sensitivity and electrophoretic mobility was found in the hybrid cells, and some of the evidence suggests that β-glucuronidase may be a polymer, and that "interspecific hybrid molecules" may be formed in these cells.

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