and less than 4 percent in phage 21. The c_I gene of λ should be similar in size to the repressor gene of phage 434, since the molecular weights are similar (*20*). At present it is difficult to speculate on the meaning of the short region of apparent homology within the unpaired $i^{434}/+$ segment, probably within gene c_I (Fig. 5B).

The distance between the right ends of the i^{434} and i^{21} substitutions permits assignment of 0.7 percent of the λ^+ length to region *y* and gene c_{II}. There is excellent agreement between the earlier mapping of the left end of gene O [79 to 81 percent from the left terminus (*16*)] and the position of the right end of the i^{21} substitution (79.8 percent from the left terminus), which is located between genes c_{II} and O (Fig. 5C).

Another conclusion to be derived from our data is the identity or near identity of the so-called b5 region in the λb5 "mutant" and the i^{21} nonhomology region in λi^{21} (*1*, *7*). Phage λb5, which has a density identical to that of phage λi^{21} and which has the immunity of phage 21 (*1*, *7*, *21*), is a recombinant between λ^+ and the descendents of a plaque which appeared as a fortuitous contaminant during crossing of λ mutants (*22*). Our data on the position of the b5 region agree well with the results of Davis and Davidson (*3*), although the length computed by them for the corresponding deletion in the λ genome is substantially lower (5.3 percent) than the value we obtained by direct measurement of the homoduplexes (Table 1 and 3) and the single-stranded λ DNA [7.6 to 9 percent (*16a*)] within the unpaired b5/+ region (Fig. 5A).

The lack of pairing between particular regions of the *l* and *r* strands in a heteroduplex could be caused either by substitution or by inversion of a segment of the genome. In the case of inversion, the base sequences of the single DNA strands in the unpaired region would be identical instead of complementary. The differences in length between the single-stranded i^{λ} segment on one hand, and the corresponding i^{434}, i^{21}, or b5 segments on the other, provide an argument against inversion; the absence of any observable homologies between the *r* strands of λ^+ and the *r* strands of the λb5 mutant is also an argument against inversion.

It can be concluded that the method described, whereby both the single-stranded and double-stranded regions of various heteroduplexes of viral DNA can be accurately measured, permits the construction of precise molecular maps, including the assignment of both position and size to various genes (*28*).

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References and Notes

1. The strains used are listed in Ref. *2*. Phage λc_{72} will be referred to as λ^+ or (+) in our report, since the electron microscopic appearance of λc_{72} DNA is indistinguishable from that of the parental ("wild type") strain λ_{papa} (*9–11*), from which it differs by a point mutation in gene c_I. In phages λb2 and λcb2, the central b2+ region has been deleted (*7*), whereas the symbols i^{21} and i^{434} indicate that a segment of the λ genome, including the so-called immunity region i^{λ}, has been deleted and replaced by analogous regions of the λ-related phages 21 or 434, respectively. Strains λi^{434} and λi^{21} were originally described as 434hy and 21hyl, respectively (*10*, *11*). As evident in this communication and from immunity studies (*9*, *21*, *22*), region b5 in phages λb5 or λb2b5 (*7*) is probably identical to i^{21}. The buoyant densities of these phages, as measured by Hradecna and Szybalski (*2*), are 1.508 g/cm (λc_{72} or λ_{papa}), 1.491 (λb2 or λcb2), 1.483 to 1.484 (λb2b5), 1.505 (λi^{434}), 1.501 (λi^{21}), and 1.483 to 1.484 ($\lambda b2 i^{21}$); see also Table 1. The symbols λ*dgal*, λ*bio*, and λ*dbio* designate defective (*d*) or plaque-forming phages in which some λ genes were replaced by *E. coli* DNA (*9*, *27*). For definition of DNA strands *l*(=W) and *r*(=C) see (*2*) and (*17*).
2. Z. Hradecna and W. Szybalski, *Virology* **32**, 633 (1967); unpublished data.
3. R. W. Davis and N. Davidson, *Proc. Nat. Acad. Sci. U.S.* **60**, 243 (1968).
4. J. A. Subirana, *Biopolymers* **4**, 189 (1965).
5. A. K. Kleinschmidt and R. K. Zahn, *Z. Naturforsch.* **14b**, 770 (1959).
6. H. Ris and B. L. Chandler, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 1 (1963).
7. G. Kellenberger, M. L. Zichichi, J. Weigle, *Nature* **187**, 161 (1960).
8. H. A. Eisen, C. R. Fuerst, L. Siminovitch, R. Thomas, L. Lambert, L. Pereira Da Silva, F. Jacob, *Virology* **30**, 224 (1966); λ genes N and c_{II} are also deleted in phage λb5 (W. F. Dove, personal communication).
9. W. F. Dove, *Ann. Rev. Gen.* **2**, 305 (1968).
10. M. Liedke-Kulke and A. D. Kaiser, *Virology* **32**, 475 (1967).
11. A. D. Kaiser, *ibid.* **3**, 42 (1957); ——— and F. Jacob, *ibid.* **4**, 509 (1957).
12. E. Burgi, personal communication.
13. J. S. Parkinson and R. W. Davis, personal communication.
14. E. Jordan, *J. Mol. Biol.* **10**, 341 (1964).
15. A. Skalka, E. Burgi, A. D. Hershey, *ibid.* **34**, 1 (1968).
16. D. S. Hogness, W. Doerfler, J. B. Egan, L. W. Black, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 129 (1966).
16a. Recent electron micrographic measurements of heteroduplexes formed between *l* strands of λi^{21} and λi^{434} DNA and *r* strands of λ*bio* phages (*1*), in which most of the λ DNA between the right end of b2+ and the left end of the i^{21} region has been replaced by *E. coli* DNA, indicate that the left end of the i^{21} substitution is located 72.2 percent from the left λ^+ terminus—that is, 1.4 percent farther to the right than the distance of 70.8 percent shown in Fig. 5c (Z. Hradecna and W. Szybalski, *Virology*, in press; D. M. Zuhse, M. J. Fiandt, Z. Hradecna, W. Szybalski, unpublished). The i^{λ} region in the $\lambda^+/\lambda b2 i^{21}$ heteroduplex (Fig. 5A) thus would have to be shortened to 7.6 percent of the λ^+ length. The difference between the lengths of the i^{λ} and i^{21} regions would then become 3.6 percent (7.6 minus 4.0), which value is in excellent agreement with the computed difference of 3.5 percent between the total lengths of the λb2/λb2 and λb2b5/λb2b5 homoduplexes [100 × (14.8 — 14.2)/17.0 = 3.5; Tables 1 and 3]. Other measurements summarized in Fig. 5C remain unaltered by the aforementioned recent studies.
17. W. Szybalski, *Can. Cancer Conf.*, in press.
18. M. Ptashne, *Nature* **214**, 232 (1967); ——— and N. Hopkins, *Proc. Nat. Acad. Sci. U.S.* **60**, 1282 (1968); mutation v_2 identifies the o_L operator and mutations $v_1 v_3$ identify the o_R operator (*17*).
19. P. D. Bear and A. Skalka, *Carnegie Inst. Wash. Year B.* **67**, 557 (1968); personal communication.
20. V. Pirrotta, personal communication.
21. S. Brenner, personal communication.
22. J. Weigle, personal communication.
23. L. A. MacHattie and C. A. Thomas, Jr., *Science* **144**, 1142 (1964).
24. L. G. Caro, *Virology* **25**, 226 (1965).
25. R. L. Baldwin, P. Barrand, A. Fritsch, D. A. Goldthwait, F. Jacob, *J. Mol. Biol.* **17**, 343 (1966).
26. E. Burgi, *Proc. Nat. Acad. Sci. U.S.* **49**, 151 (1963).
27. B. Westmoreland, thesis, University of Wisconsin (1968).
28. Supported in part by NSF grants RG4738 and GB-2096 and by NIH grant GM 4738. We thank Drs. Z. Hradecna and A. Guha for the separated strands of λ DNA and Miss Elaine C. Babitz for measuring the contour length of the DNA molecules. We also thank Drs. J. Weigle, N. Davidson, and A. D. Hershey for helpful information and for reading this manuscript. Dr. N. Davidson pointed out to us that, in 1961, M. Nomura and S. Benzer [*J. Mol. Biol.* **3**, 684 (1961)] proposed the mapping of deletions by the electron microscopy of heteroduplexes, whereas our study was initiated only in 1962 by B.W. (*27*).

2 December 1968

Ontogeny of Soluble and Mitochondrial Tyrosine Aminotransferases

Abstract. *The development of the soluble and mitochondrial forms of tyrosine aminotransferase was observed in fetal and neonatal rhesus monkey tissues. The mitochondrial activity is detectable in early fetal life; the soluble form reaches significant activity just before the birth of the animal.*

The mitochondrial form of tyrosine aminotransferase (E.C. 2.6.1.5) (TAT) which differs from the soluble form of this enzyme (*1*) was first detected in a liver biopsy from a patient with tyrosinemia who lacks the soluble enzyme (*2*). Mitochondrial TAT activity has been found in most of the tissues examined from adult rats, goats, monkeys, and humans. Because the transitory tyrosinemia observed in 89 percent of premature infants (*3*) has been attributed in part to low amounts of this enzyme (*4*), we investigated the time of appearance and development of both the soluble and mitochondrial forms. The results suggest that the control of tyrosine metabolism in the mammalian fetus may closely resemble that of the

patient who lacks the soluble enzyme activity.

Livers were obtained from fetal rhesus monkeys (*Macaca mulatta*) at Cesarian section during the latter half of gestation, or by biopsy from the newborn and from pregnant and nonpregnant adults. All tissues were kept at 4°C and were processed within 2 hours of sampling. Subcellular fractionation was carried out (*5*), and soluble and mitochondrial fractions were assayed radiochemically (*1*). The results (Fig. 1) reveal significant activity of the mitochondrial, but very little activity of soluble tyrosine aminotransferase, at 75 and 100 days of gestation. The 100-day-old monkey fetus is at a stage of development equivalent to that of the human fetus at 7 months gestation (*6*). During the period just preceding birth, which occurs at 168 ± 4 days after conception, the specific activity of soluble TAT progressively increased from 3 mmole of *p*-hydroxyphenylpyruvate (*p*HPP) per milligram per 30 minutes at 100 days to 6 mmole at 152 days, and 17 mmole and 12 mmole in the fetuses killed at 153 and 162 days. The combined values from individual animals (Fig. 1) indicate that increase in soluble TAT occurs just before birth of the animal and is not dependent on the birth process. The increase in activity of the soluble form of the enzyme continues in the early neonatal period. The specific activity of the mitochondrial form shows a two- to threefold increase during the postnatal period. A similar reversal from predominantly mitochondrial enzyme at 18 days to predominantly soluble TAT activity at 20 days gestation was found in pooled samples of fetal rat livers.

The transition from a low to a high activity of soluble TAT during late fetal life or at the time of delivery is consistent with the findings reported for livers from premature and neonatal humans (*4*), rats (*7*), and rabbits (*8*). Inasmuch as the mitochondrial enzyme is the major form during earlier periods of gestation, it may also be the important site of regulation of tyrosine metabolism in the developing fetus. The contribution of mitochondrial transamination to tyrosine metabolism in the fetus has not been considered until now. By analogy with the alterations encountered in the patient who lacks soluble TAT, steady-state amounts of both tyrosine and *p*HPP would be expected to be elevated until such time as the soluble tyrosine oxidizing system has developed, that is, at about birth. The contribution of the increase in mitochondrial transamination after birth is unknown.

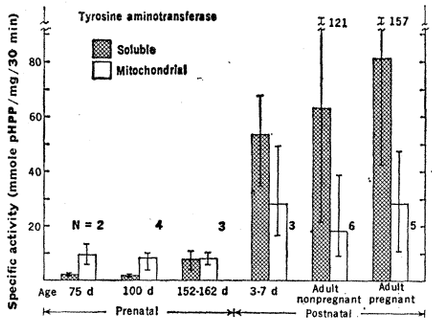

Fig. 1. Tyrosine aminotransferase in rhesus monkey livers during prenatal and postnatal development. Results are expressed as mean and observed range of specific activity (mmole of *p*HPP produced, per milligram of protein per 30 minutes) for soluble and mitochondrial forms (*1*). The standard deviation of the method at the low-enzyme activities measured during the prenatal period is 3 mmole. Values for soluble TAT at 75 and 100 days are not significantly different from zero. The lowest value for mitochondrial activity at both 75 and 100 days is at the 93rd percentile of the *t* distribution.

Amounts of tyrosine in serums from rhesus monkey fetuses at 152 and 153 days of gestation were 0.562 and 1.667 mg per 100 ml, compared to 0.217 and 0.435 mg per 100 ml in the maternal serum (*9*). Amniotic fluid obtained at 75 and 161 days of gestation contained 0.062 mmole and 0.120 mmole of *p*HPP per liter (*10*), an indication that tyrosine is catabolized during intrauterine life.

The two subcellular pathways for tyrosine metabolism are illustrated in Fig. 2. During the prenatal period before significant soluble TAT activity appears, only the mitochondrial pathway would operate. It differs from the soluble pathway in two ways: no *p*HPP hydroxylase is present in mitchondria (*11*), and glutamate, which inhibits both forms of TAT, does not accumulate. Regeneration of α-ketoglutarate from glutamate by glutamate dehydrogenase was demonstrated in mitochondria from adult rat liver samples (*1*). Glutamate dehydrogenase was also found in mitochondria from rhesus fetal livers as early as the 75th day of gestation (*12*).

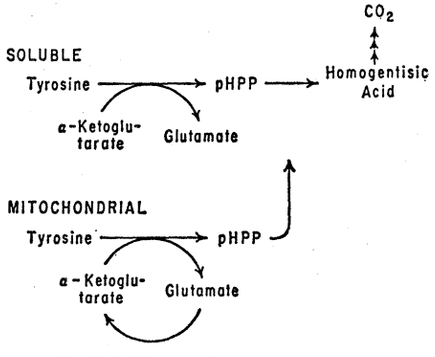

Fig. 2. Soluble and mitochondrial pathways of tyrosine metabolism.

Factors which predispose to tyrosinemia and tyrosyluria in the newborn include prematurity and a diet which is high in protein and low in ascorbic acid. Therapy with large doses of ascorbic acid completely reverses the metabolic abnormalities (*13*), and no permanent residua have been found in the subsequent development of such infants (*14*).

Postnatal development of TAT and activation of *p*HPP hydroxylase by ascorbic acid may account for the transitory nature of this defect (*4*). The time of appearance of the soluble enzyme coincides with birth in rats and rabbits (*7, 8*). The late prenatal rise in rhesus monkey fetuses suggests that the factors responsible for induction and repression of soluble TAT may be related to gestational age in primates. These include the hormones, hydrocortisone (*15*), growth hormone (*16*), glucagon, insulin (*17*), and other less direct effectors such as blood glucose, epinephrine, and adenosine-3′,5′-cyclic phosphate (cyclic AMP) (*18*). The mitochondrial form is responsive to hydrocortisone induction in adult rat liver (*1*).

Except in the known inborn errors of tyrosine metabolism (*1, 19*), significant deviations from normal steady-state amounts of tyrosine and *p*HPP occur rarely in postnatal life. McCann *et al.* (*20*), described slight elevations of *p*HPP in urine from patients with connective tissue disease or liver disease. An understanding of both normal and defective tyrosine metabolism must take cognizance of the existence of the two tyrosine metabolizing systems.

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References and Notes

1. J. H. Fellman, P. J. Vanbellinghen, R. T. Jones, R. D. Koler, *Biochemistry*, in press.
2. R. A. Campbell, N. R. M. Buist, E. Y. Jacinto, R. D. Koler, F. Hecht, R. T. Jones, *Program*, 37th Annual Meeting, Society for Pediatric Research, 1967.
3. J. H. Menkes and M. E. Avery, *Bull. Johns Hopkins Hosp.* **113**, 301 (1963).
4. N. Kretchmer, *Pediatrics* **23**, 606 (1959).
5. G. H. Hogeboom, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955), vol. 1, p. 16.
6. R. E. Behrman, A. E. Seeds, Jr., F. C. Battaglia, A. E. Hellegers, P. D. Bruns, *J. Pediat.* **65**, 38 (1964).
7. F. Sereni, F. T. Kenney, N. Kretchmer, *J. Biol. Chem.* **234**, 609 (1959).
8. G. Litwack and A. M. Nemeth, *Arch. Biochem. Biophys.* **109**, 316 (1965).
9. Automatic amino acid analysis was done with the use of a Spinco 120 analyzer (Spinco-Beckman, Palo Alto, Calif.) according to the procedure of S. Moore, D. H. Spackman, W. H. Stein [*Anal. Biochem.* **30**, 1185, (1958)]. Similar observations have been reported by Q. Q. Kerr and S. S. Waisman [in *Amino Acid Metabolism and Genetic Variation*, W. L. Nyhan, Ed. (McGraw-Hill, New York, 1967), p. 429].
10. Measurement of *p*HPP was done colorimetrically (*1*) on deproteinized amniotic fluid. The values obtained could reflect keto acids other than *p*HPP.
11. J. H. Fellman and P. J. Vanbellinghen, unpublished results.
12. Glutamate dehydrogenase was done on the mitochondrial fractions after disruption by sonic vibration according to the method of H. J. Strecker, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955), vol. 2, p. 220.
13. S. Z. Levine, H. H. Gordon, E. Marples, *J. Clin. Invest.* **20**, 209 (1941).
14. J. H. Menkes, V. Chernick, B. Ringel, *J. Pediat.* **68**, 583 (1966).
15. C. C. Lin and W. E. Knox, *J. Biol. Chem.* **233**, 1186 (1958).
16. F. T. Kenney, *ibid.* **242**, 4367 (1967); F. Labrie and A. Korner, *ibid.* **243**, 1120 (1968).
17. O. Greengard and H. K. Dewey, *ibid.* **242**, 2986 (1967); D. D. Holten and F. T. Kenney, *ibid.* p. 4372; W. D. Wicks, *ibid.* **243**, 900 (1968).
18. W. D. Wicks, *Science* **160**, 997 (1968).
19. B. N. LaDu, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, Eds. (McGraw-Hill, New York, 1966), p. 295.
20. D. S. McCann, J. G. Mitchell, M. K. Keech, A. J. Boyle, *J. Chronic Dis.* **20**, 781 (1967).

21. Supported by PHS grants CA 07941, NINDB 01572, NICHD 02757, and NICHD 01901. Publication No. 1234 from the Oregon Regional Primate Center which is supported in part by PHS grant FR 00163. We thank Marie Duerst for technical assistance.

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Conductivity and Photoconductivity in Egg White

Abstract. *Activation energies of 1.47 to 2.94 electron volts were obtained in egg white. These values are too large to be consistent wtih the large observed currents if one uses a semiconductor model and indicate that the standard semiconductor model is inappropriate for this biological material.*

The values of electrical resistivity and activation energy of liquid and crystalline albumin found by different investigators (*1*, *2*) vary considerably. Although it is not pure albumin, egg white upon being dried becomes a hard, clear, amber-colored solid that can be handled easily. Conductivity studies on it encounter neither the barrier problems associated with powders nor the electrode problems associated with solutions, and, except for being heated and dried, the natural substance is not treated in any way. We therefore investigated some of the electrical properties of the material in the belief that they might prove a useful adjunct to the findings for albumin, by serving as a bridge to values for a biological material in the natural state.

Samples were prepared from chunks of egg white that had been boiled in water for 15 minutes and dried in air for several days. The hard, translucent solid of less than half its original volume was painted on two sides with silver epoxy, to which leads were attached. A guard ring encircling the remaining sides prevented surface currents from being recorded. A Chromel-constantan thermocouple was cemented to the guard ring with insulating epoxy.

For potentials under 1.5 volts a battery and potentiometer were used; for potentials between 1.5 and 100 volts a high-voltage supply was used. Currents were measured with a picoammeter. Temperature was varied with a thermoelectric heater-cooler attached to two copper rods that held the sample on the sides with electrodes, the latter being electrically insulated from the rods. The sample was contained in a light-tight box and could be illuminated through an optical shutter by a 100-watt mercury lamp, Corning glass filters isolating different regions of the spectrum.

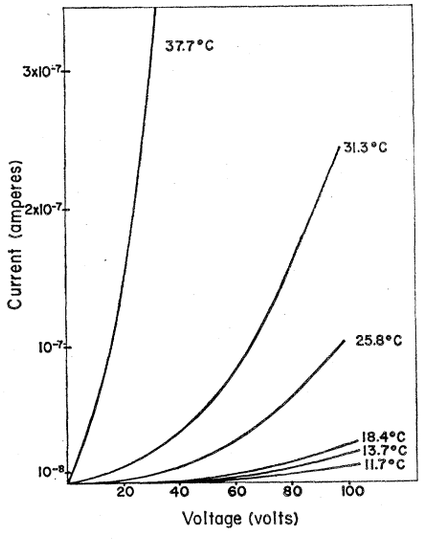

Fig. 1. Current as a function of voltage at different temperatures for a sample 0.8 by 0.8 by 0.5 cm thick.

The absorption spectrum, measured with a Cary-14 spectrophotometer, was essentially flat over the visible and near-ultraviolet range. For a sample thickness of several millimeters, radiation from the untraviolet to 5500 Å is absorbed totally. From 5500 Å to longer wavelengths 1 or 2 percent is transmitted, accounting for the sample's translucency and yellow color.

Curves of dark current as a function of voltage, at different temperatures, for a sample 0.8 by 0.8 by 0.5 cm thick, are shown on a linear scale in Fig. 1. The current is not ohmic; it depends roughly on the square of the voltage. Figure 2 shows the current as a function of the reciprocal temperature on a semilogarithmic scale. There seem to be several slopes, the steepest at higher temperatures, yielding an activation energy ΔE equal to 2.94 ev in the expression

$$i = i_0 \, e^{-\Delta E/KT}$$

where i is the current density, i_0 is a constant, K is Boltzmann's constant, e is the exponential, and T is the absolute temperature. The middle range yields a slope corresponding to ΔE of 1.47 ev. Although at lower temperatures the slope seems to flatten and then become steeper, a single slope has been drawn. The behavior in this last range is shown more clearly in Fig. 3, where the logarithm of the current, at 1 volt, is plotted against the reciprocal temperature.

When the current is proportional, not to the voltage, but to some higher power of the voltage, an activation energy may still be relevant when the same activation energy is applicable to all voltages. It is possible, in that case, that the origin of the voltage dependence is not related to the source of current but is an independent phenomenon.

What emerges from these measurements are, first, large activation en-