tativ bedeutendste Metabolit des Dopamins anzusehen ist, kann als nicht ganz geklärt bezeichnet werden; im Striatum findet die O-Methylierung des DOPAC zu HVA in stärkerem Mass statt als im mesolimbischen System[12]; zudem interessierte als Effektor nur die letztere Verbindung, da die Feedback-Hemmung der Tyrosin-Hydroxylierung durch Catecholverbindungen gut bekannt ist[8, 15, 16]. Wie aus der Abbildung zu ersehen ist, ist der Effekt der HVA auf die Aktivität der TH bis zu einer Konzentration von 10^{-4} M/l dosisabhängig; zwischen der genannten Konzentration und 10^{-5} M/l bildete die Aktivierungskurve ein Plateau. Wenngleich der Effekt für das letztere Probenkollektiv statistisch nicht mehr als signifikant zu werten war, ist aufgrund des Kurvenverlaufs, der gerade vor Erreichen der endogenen Konzentration noch eine deutliche Wirkung erkennen lässt, dennoch eine Bedeutung auch der Ergebnisse nach Einsatz von 10^{-5} M HVA/l nicht auszuschliessen.

Im Rahmen einer Effektoren-Übersicht untersuchten McGeer et al.[8] auch die Wirkung der HVA auf die Aktivität der Tyrosinhydroxylase. Es wurden Werte, die innerhalb einer Streuung von ± 15% um die Kontrollen geschart waren, beobachtet und als unsignifikant gewertet. Die endogene Konzentration an HVA aus dem Homogenat des Rattenstriatums liegt bei $2{,}5 \times 10^{-6}$ M/l[13]; an den dopaminergen Neuronen selbst dürfte die Konzentration von Transmitter und Metabolit einen beträchtlich höheren Wert erreichen: Haug[17] postuliert ein Ansteigen des Verhältnisses von Nervenzellvolumen: Gesamtvolumen im Laufe der Phylogenese; da das als Nager der Ratte phylogenetisch verwandte Kaninchen einen Wert von 1:20 aufwies, ist anzunehmen, dass der Quotient für die Ratte in mindestens eben dieser Grössenordnung liegt. Es kann somit eine Bedeutung des beschriebenen Effektes auch in vivo erwogen werden, da als wahrscheinlich angenommen werden kann, dass die Konzentration an HVA an dopaminergen Neuronen jenen Wert erreicht, der in den beschriebenen Versuchen noch eine deutliche Aktivierung erkennen liess. Die regulatorische Bedeutung des beobachteten Effektes könnte darin liegen, dass die Catecholamin-Synthese durch eine Akkumulation eines Hauptmetaboliten verstärkt wird, da diese Transmittererfordernis signalisiert.

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Phosphonacetyl-L-aspartate: An aspartate transcarbamylase inhibitor causing larval death and rudimentary wing phenocopies in Drosophila melanogaster

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Summary. Phosphonacetyl-L-aspartate, an inhibitor and transition state analogue of aspartate transcarbamylase, has been found to cause rudimentary wing phenocopies and larval death in Drosophila melanogaster. We believe this compound may prove useful as a selection agent for regulatory mutations at the rudimentary locus.

The need to use regulatory mutants in studying gene regulation is widely recognized. In eukaryotes, especially multicellular ones, obtaining such mutants is difficult. We are currently engaged in screening for regulatory mutants at the rudimentary wing (r) locus in Drosophila melanogaster. This locus is believed to code for the first 3 enzymes in the pyrimidine biosynthetic pathway, carbamyl phosphate synthetase, aspartate transcarbamylase (ATCase) and dihydroorotase[2–5]. Rudimentary mutants require pyrimidines for larval development and female fertility and have changes in the wings of both sexes ranging from a slight irregularity in the marginal wing bristles to short, blistered wings with very sparse marginal bristles[6, 7]. We are attemting to recover mutants which synthesize excess quantities of the first 3 enzymes in pyrimidine biosynthesis. Such 'overproducers' might be recovered by selecting for mutants resistant to compounds which inhibit pyrimidine biosynthesis. In microorganisms one such compound, 6-azauracil (6-AU), when converted to 6-azauridine monophosphate (6-AUMP), is known to be a competitive inhibitor of orotidine monophosphate decarboxylase, the terminal enzyme in pyrimidine biosynthesis[8]. 6-AU kills Drosophila larvae and produces r phenocopies[9]. This report concerns a second compound, phosphonacetyl-L-aspartate (PALA), an inhibitor and transition state analogue of ATCase[10]. PALA inhibits ATCases from bacteria[10], mammals[11] and Drosophila[12] and kills mammalian cells in culture[13]. We report here that PALA induces r phenocopies and kills wild-type larvae.

Materials and methods. Na_4PALA was synthesized according to Stark and Swyryd[14]. An Oregon r stock was used. The defined media of Sang[15] as modified by Falk and Nash[16] was used without RNA except as noted. Eggs were collected on standard media over a 24-h-period. The resulting eggs (and some first instar larvae) were recovered and rinsed thoroughly with water. They were then floated in 20% sucrose to remove food and agar and

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again throroughly rinsed and blotted dry. Approximately 100 eggs, determined by weight were placed in a vial on 5 ml of medium. The number of flies eclosing/vial was determined at intervals after 2 weeks's incubation at 25 °C until no more flies eclosed.

Results and discussion. The number of flies eclosing in control vials varies from approximately 30/vial to 60/vial with a mean of about 50/vial. The table shows that concentrations of PALA up to 10^{-3} M have no effect on viability. At concentrations between 10^{-3} M and 2.5×10^{-2} M PALA the number of flies eclosing decreases so that no flies eclose at 2.5×10^{-2} M. Approximately 40% of flies eclosing at 10^{-2} M are rudimentary phenocopies ranging from mild to very severe (figure). The lethality of 6-AU is first detected at 10^{-5} M. At 2.5×10^{-3} M virtually no flies eclose and at 5×10^{-3} M lethality is 100%. An ideal selection agent should be specific in its mode of inhibition. In this case the agent should only affect pyrimidine synthesis and pyrimidines should reverse the effects of the inhibitor. Accordingly, we tested the effect of addition of pyrimidine or purine nucleosides or RNA to media containing 5×10^{-3} M PALA or 2.5×10^{-3} M 6-AU, concentrations of the respective inhibitors which give almost complete lethality. As seen in the table addition of pyrimidine nucleosides or RNA causes reversal of PALA and 6-AU inhibition. The addition of purine nucleosides has no effect on lethality.

These preliminary results indicate that PALA is lethal to larvae and can be used in a selection scheme designed to recover resistant mutants. Further, PALA seems to have several advantages over 6-AU as a selection agent. In bacterial systems a major class of 6-AU resistant mutants results from loss of ability to change 6-AU to 6-AUMP[8]. However, PALA need not be metabolized for ATCase inhibition and thus such a class of mutants should not be found using PALA. Also, it appears that there is very little metabolism of PALA[17]. Another advantage of

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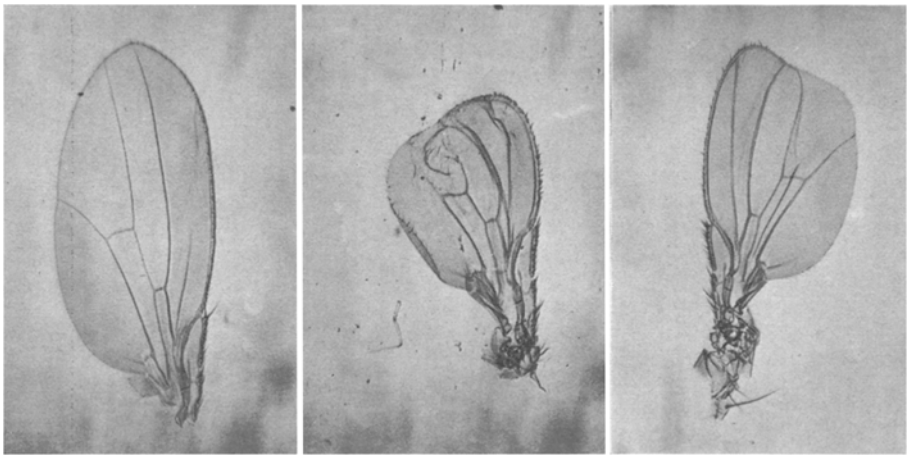

Phenocopy of rudimentary wing in Drosophila melanogaster larva fed defined media containing 10^{-2} M PALA. *A* Wild type wing. *B* Typical rudimentary wing. *C* PALA phenocopy of rudimentary wing.

Lethal effects of PALA and 6-AU on wild-type larvae

Concentration PALA (M)	Average No. flies exclosing/vial ± 1 SD	Average No. flies eclosing/vial expressed as percent of control ± 1 SD	Concentration 6-AU (M)	Average No. flies eclosing/vial ± 1 SD	Average No. flies eclosing/vial expressed as percent of control ± 1 SD
0 (control)	47.6 ± 13.3	100 ± 28	0 (control)	49.3 ± 8.8	100 ± 18
0 + C	49.5 ± 15.2	104 ± 32	0 + C	46.8 ± 13.8	95 ± 28
0 + A	40.5 ± 17.6	85 ± 37	0 + A	47.3 ± 14.3	96 ± 29
10^{-4}	34.4 ± 13.8	72 ± 29	10^{-5}	27.2 ± 9.9	55 ± 20
10^{-3}	37.2 ± 8.3	78 ± 17	10^{-4}	15.9 ± 5.5	32 ± 11
5.0×10^{-3}	4.4 ± 2.2	9 ± 5	5.0×10^{-4}	7.6 ± 2.4	15 ± 5
10^{-2}	1.8 + 0.8	4 ± 2	10^{-3}	4.2 ± 3.2	8 ± 6
2.5×10^{-2}	0	0	2.5×10^{-3}	0.1 ± 0.3	0 ± 1
5.0×10^{-2}	0	0	5.0×10^{-3}	0	0
			7.5×10^{-3}	0	0
			10^{-2}	0	0
5.0×10^{-3} + C	45.2 ± 10.0	95 ± 21			
5.0×10^{-3} + U	39.0 ± 10.9	82 ± 23	2.5×10^{-3} + C	32.0 ± 15.3	65 ± 31
5.0×10^{-3} + A	3.8 ± 4.8	8 ± 10	2.5×10^{-3} + U	23.7 ± 12.3	48 ± 25
5.0×10^{-3} + G	4.3 ± 3.8	9 ± 8	2.5×10^{-3} + A	1.5 ± 1.0	3 ± 2
5.0×10^{-3} + R	47.1 ± 15.7	99 ± 33	2.5×10^{-3} + G	0.5 ± 1.0	1 ± 2
			2.5×10^{-3} + R	51.3 ± 6.9	104 ± 14

Procedures are given in 'materials and methods'. 9 replicates were done for each experimental condition. Reversal of lethality was tested by the addition of 2.5×10^{-2} M pyrimidine or purine nucleosides (C-cytidine, U-uridine, A-adenosine, G-guanosine) or 1% RNA (R) to a lethal concentration of PALA (5×10^{-3} M) or 6-AU (2.5×10^{-3} M).

PALA is that it inhibits an enzyme coded for at the locus under study and should not select for overproducers of enzymes catalyzing later steps in the pathway which 6-AU might do. 6-AU could be used as a secondary selection agent in retesting and maintaining stocks of PALA resistant flies. However, the most important advantage of PALA stems from the fact that it is a transition state analogue of ATCase. Mutations leading to modification of ATCase so that it does not interact with PALA will probably also eliminate ATCase activity. Increased ATCase levels thus are a likely means for obtaining resistance to PALA. In conclusion, these preliminary results indicate that PALA could be an excellent selection agent for overproducers.

The nucleolar chromosome in embryos of Rana pipiens[1]

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Summary. Karyotype analyses of prometaphases from medullary plate cells derived from mid-neurulae of Rana pipiens have led to the identification of the nucleolar chromosome and nucleolar organizing region, which is located on the longer arm of a small sub-metacentric chromosome (No. 10).

Nucleolar chromosomes have been identified in several systems. Direct identification has been made in pachytene chromosomes, e.g.[4] and occasionally in late prophases and early prometaphases of somatic cells when the nucleoli persisted[5]. Both approaches to identifying the nucleolar chromosome of an organism are extremely time consuming and difficult due to the lack of spreading of the chromosomes at these stages and also due to the rarity of persistent nucleoli in mitotic chromosomes. Attempts to induce the persistence of nucleoli in mitotic chromosomes have been reported[6,7]; however, these approaches have not yielded wide spread application in different systems. It is also possible to identify nucleolar chromosomes in mitotic chromosomes through an indirect approach. For example, in the Xenopus mutant, heterozygous for the nucleolus, one can correlate the absence of a prominent secondary constriction in one homologue of a pair of metaphase chromosomes with the absence of one nucleolus in interphase nuclei[8,9]. We report here the direct identification of the nucleolar chromosome and the nucleolar organizing region in embryos of Vermont Rana pipiens, revealed microscopically in prometaphases of medullary plate cells containing a large number of persistent nucleoli.

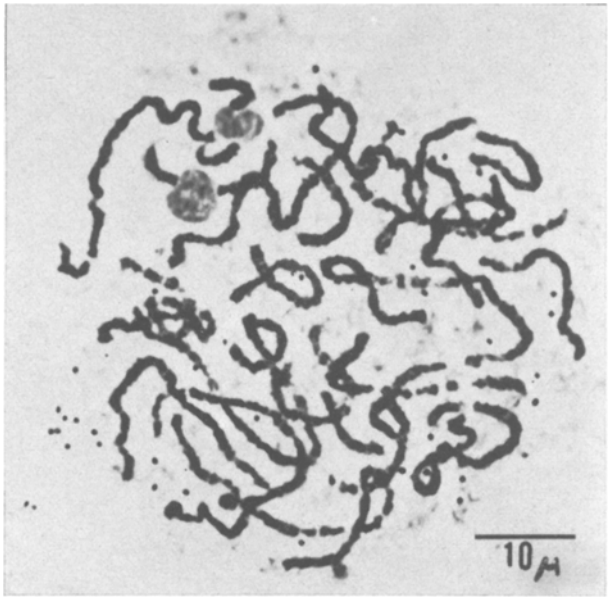

Fig. 1. Prometaphase from a medullary plate cell derived from a diploid embryo at the mid-neurula stage. 1 pair of small homologous chromosomes contains 2 nucleoli.

Materials and methods. Adult Rana pipiens used in these studies were purchased from J. M. Hazen Co., Alburg, Vermont, and are derived from 5 different shipments obtained over a period of 4 years. Ovulation was induced by intraabdominal injection of pituitary glands and artificial insemination was performed with sperm suspensions derived from macerated testes, based on original procedures of Rugh[10]. When the embryos attained the stage of mid-neurula (stage 14[11]), the medullary plate along with the underlying mesoderm and archenteron roof was excised in Steinberg's salt solution[12]. Next, the medullary plate was either mechanically separated from the underlying tissues in Steinberg's solution, or transferred to 0.5% trypsin dissolved in modified Steinberg's solution[13] for 2–3 min, during which time the medullary plate was separated from the other tissues. The medullary plate was then rinsed in Steinberg's solution for 2–3 min, and immediately fixed and stained in acetic orcein. Subsequently, the tissue was squashed and processed for permanent preparations.

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