ing (Fig. 3). Under conditions in which the substrate is scarce, it is advantageous for the cell to increase the production of enzyme to a maximum so that even at partial saturation an adequate input is maintained. However, unless there is a kinetic feedback control, the high level of the kinase may at times cause over-production of L-α-glycerophosphate, thereby retarding growth (*10*). Thus, if the glycerol concentration in the environment suddenly rises, from 10^{-6} to $10^{-5}M$, flooding with the phosphorylated product may occur before a corrective effect by repression can be expressed. The liability of producing derepressed levels of the desensitized kinase in fact could be shown with cells of strain 43 growing on succinate. The addition of glycerol to such a culture severely impeded growth. Cells making the wild-type glycerol kinase did not exhibit this vulnerability.

The establishment of FDP as the kinetic regulator of glycerol kinase allows momentary carbon surplus derived from glycerol to be deposited in the hexose diphosphate pool, since both triosephosphate isomerase and aldolase are either constitutive or internally induced (*11*), and the equilibrium is much in favor of the synthesis of FDP from the triosephates (*12*).

The inhibition of the kinase by a glycolytic intermediate provides an additional means of excluding glycerol utilization during glucose metabolism. The efficiency of this exclusion is made all the more powerful by the fact that the product of the kinase reaction is the inducer of the enzyme (*13*).

The finding that remote-product inhibition of the first enzyme in a dissimilatory pathway illustrates further the similarity of regulatory mechanisms of anabolic, amphibolic, and catabolic systems in which feedback control can be exercised during enzyme action as well as enzyme synthesis (*14*).

N. ZWAIG
E. C. C. LIN
Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts

References and Notes

1. H. E. Umbarger, *Science* **123**, 848 (1956); R. A. Yates and A. B. Pardee, *J. Biol. Chem.* **221**, 757 (1956); M. S. Brooke, D. Ushiba, B. Magasanik, *J. Bacteriol.* **68**, 534 (1954); R. B. Roberts, P. H. Abelson, D. B. Cowie, E. T. Bolton, R. J. Britten, *Carnegie Inst. Wash. Publ.* No. 607 (1957); G. N. Cohen, *Ann. Rev. Microbiol.* **19**, 105 (1965).
2. J. M. Ashworth and H. L. Kornberg, *Biochim. Biophys. Acta* **73**, 519 (1963).
3. E. C. C. Lin, J. P. Koch, T. M. Chused, S. E. Jorgensen, *Proc. Nat. Acad. Sci. U.S.* **48**, 2145 (1962).
4. S. Hayashi and E. C. C. Lin, *Biochim. Biophys. Acta* **94**, 479 (1965).
5. J. P. Koch, S. Hayashi, E. C. C. Lin, *J. Biol. Chem.* **239**, 3106 (1964).
6. N. Zwaig and E. C. C. Lin, *Biochem. Biophys. Res. Commun.* **22**, 414 (1965).
7. S. Hayashi and E. C. C. Lin, in preparation.
8. J. Monod, J. Wyman, J. P. Changeux, *J. Mol. Biol.* **12**, 88 (1965).
9. A. Böck and F. C. Neidhardt, *J. Bacteriol.*, in press.
10. N. R. Cozzarelli, J. P. Koch, S. Hayashi, E. C. C. Lin, *ibid.* **90**, 1325 (1965).
11. S. Hayashi, unpublished observations.
12. W. J. Rutter, in *The Enzymes*, P. D. Boyer, H. Lardy, K. Myrbäck, Eds. (Academic Press, New York, 1961), vol. 5, p. 341.
13. S. Hayashi and E. C. S. Lin, *J. Mol. Biol.* **14**, 515 (1965).
14. F. Jacob and J. Monod, *ibid.* **3**, 318 (1961); F. C. Neidhardt and B. Magasanik, *J. Bacteriol.* **73**, 253 (1957); B. Magasanik, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 249 (1961); E. McFall and J. Mandelstam, *Biochem. J.* **89**, 391 (1963).
15. N. R. Cozzarelli, thesis, Harvard University, 1966.
16. We thank H. Paulus, J. Koch, and N. Cozzarelli for help in the preparation of this manuscript. N.Z. is fellow of the Universidad de Buenos Aires, Republica Argentina. E.C.C.L. is supported by a USPHS career development award. Aided by grants NSF (GB-3527) and USPHS (GM-11983).

27 April 1966

Dieldrin: Interaction with Nerve Components of Cockroaches

Abstract. *We have evidence that the nerve components of the dieldrin-resistant German cockroach have less binding capacity for dieldrin than those of the susceptible cockroach; the highest interstrain difference was in the crude-nucleus fraction. The dieldrin-nerve complexes are not extracted by many organic solvents.*

Since Busvine (*1*) discussed resistance to dieldrin, as distinct from other resistance phenomena such as DDT resistance, on the basis of symptomology and cross resistance in the housefly, the problem of dieldrin-resistance has been constantly investigated (*2*). The problem has been a delight for geneticists, who discovered a simple, straightforward pattern of inheritance (*3*), and a nightmare for biochemists, who found no significant interstrain differences in the insect's defense mechanisms such as biochemical detoxication, penetration through the cuticle and the nerve sheath, storage, and excretion (*4*). Dieldrin is generally very stable and not readily detoxified by insects, though one exception was recently reported (*5*).

The only direct relation of the action of dieldrin to the central nervous system was observed by Yamasaki and Narashashi (*6*), who discovered that dieldrin-poisoned nerves of the American cockroach (*Periplaneta americana*) showed spontaneous bursts of action potential; they also noticed that the nervous systems of resistant houseflies showed much longer latent periods between the application of dieldrin and the appearance of discharge than the systems of susceptible flies (*7*).

A recent hypothesis attempted to explain the mode of action of DDT on the basis of formation of charge-transfer complexes by nerve components and DDT (*8*). The first step of this charge-transfer process was exemplified by formation of bound DDT, which apparently could be clearly distinguished from free DDT by means of molecular filtration (Sephadex colmun chromatography). This approach to the problem of an insecticide-binding mechanism may be generally applicable throughout the whole group of chlorinated hydrocarbon insecticides, including dieldrin and DDT. The implication is simple: if such a mechanism is directly implicated in dieldrin poisoning, nerve components of dieldrin-resistant individuals should clearly show a different pattern of binding with dieldrin.

The head parts from three strains of the German cockroach (*Blattella germanica* L.) were homogenized, with small Teflon Potter-Elvehjem homogenizers, in 0.25*M* sucrose solution at 0°C at a concentration of three heads per milliliter. The head samples from each strain were carefully weighed to insure equality of the homogenate concentrations among the strains; London, Fort Rucker (both resistant), and CSMA (susceptible) strains averaged 2.517, 2.508, and 2.458 mg, respectively.

In terms of LT_{50} (time to 50-percent mortality; *9*) (at 1 mg of dieldrin per jar having an inner surface of 200 cm^2), Fort Rucker (15 hours) and London strains (45 hours) were four and eleven times more resistant, respectively, than the CSMA strain (4 hours). The C^{14}-dieldrin in either absolute ethanol or acetone was added to the brain homogenate to make the final concentration $1 \times 10^{-5}M$ (final ethanol or acetone concentration, 1 percent) in a 20-ml vial with a screw cap; the system was maintained at 24°C for 1 hour. The reaction was stopped by transferring the vial to an ice bath. The resulting solution was poured into a Sephadex G-50 (medium) column of 1 by 10 cm, and each component was eluted carefully with distilled water;

Table 1. Distribution of dieldrin in certain subcellular fractions from homogenate of cockroach brain. The results are expressed as percentages of added dieldrin that were recovered from the fractions; each value is the average of four to six determinations. All t-values (in parentheses) are based on the susceptible strain.

Fraction			Resistant strains (%)		Susceptible CSMA (%)
Identity	Centrifugation		London	Fort Rucker	
	Rate ($\times g$)	Time (min)			
Crude nucleus	650	10	23.6 ± 1.2 (t_{10}, 2.39)*	25.5 ± 4.6 (t_{10}, 1.48)	35.3 ± 4.8
Mitochondrial	8,000	10	17.9 ± 4.6 (t_8, 0.40)	17.0 ± 3.6 (t_8, 0.64)	20.2 ± 3.6
Microsomal	20,000	120	1.9 ± 0.9 (t_8, 0.54)	3.5 ± 0.7 (t_8, 1.04)	2.5 ± 0.7
Supernatant	20,000	120	56.7 ± 5.8 (t_{10}, 1.83)	54.9 ± 5.3 (t_{10}, 1.67)	42.6 ± 5.1

* Significant at the 5-percent level.

2-ml fractions were collected and 0.5-ml portions were assayed by liquid-scintillation counting.

The resulting chromatograms indicated three major radioactive peaks that were associated with proteinaceous materials, nonproteinaceous organic matter, and free dieldrin (designated peaks 1, 2, and 3, respectively). The amount of radioactivity per unit protein recovered in peak 1 was highest in the London strain and lowest in the susceptible strain, the average ratio of resistant homogenates to the susceptible homogenates being 1.50 and 1.10 for London and Fort Rucker strains, respectively (average of three to four experiments). No such interstrain differences were observed among the chromatograms of peaks 2 and 3; results from the Sephadex columns are not, however, conclusive because quantitative reproducibility was very low: even with the carefully controlled method of homogenization, the height of peak 1 tended to fluctuate from one run to another.

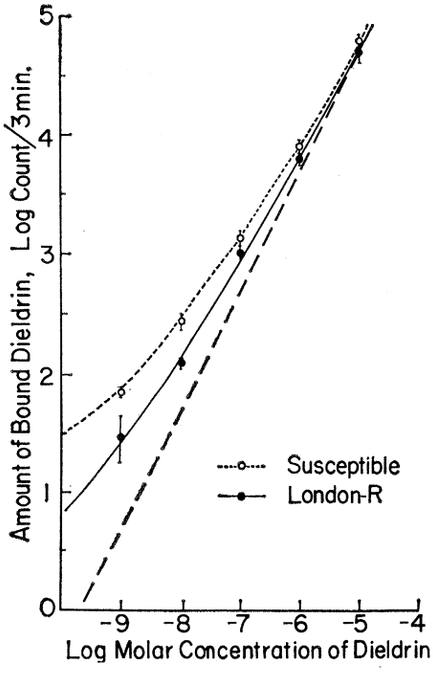

Fig. 1. Binding of C^{14}-dieldrin with the nerve components of susceptible and resistant German cockroaches. The straight line indicates the rate of a theoretical binding estimated by nonspecific absorption of dieldrin at high concentrations. Vertical lines represent standard errors of the experimental data.

To assess the binding capacity of dieldrin to subcellular components other than the soluble ones, the dieldrin-treated homogenates were each separated into four fractions by means of centrifugation and then washed twice with fresh sucrose solution; bound dieldrin was measured in terms of radioactivity recovered from each fraction (Table 1). It was found that (i) the crude-nucleus fractions from the susceptible strain absorbed more dieldrin than those from resistant strains, and (ii) the rates of dieldrin recovery from the resistant supernatants were much higher than those from the susceptible counterparts. The supernatant fraction may contain unknown amounts of free dieldrin molecules that should be extractable by any organic solvent upon partitioning. The aqueous phases of the resistant strains had higher radioactivity than those of the susceptible strain. Partitioning the supernatants with n-pentane transferred 69.3 ± 10.0 (for London), 65.5 ± 3.5 (for Fort Rucker), and 63.2 ± 5.3 percent (for CSMA) of radioactivity into the solvent phase; similar treatment with n-butanol extracted 90.0 ± 0.3 (London), 88.2 ± 1.2 (Fort Rucker), and 86.1 ± 3.8 percent (CSMA) of the total radioactivity.

Experiments with chloroform and benzene confirmed this tendency for the resistant supernatant to have more solvent-extractable dieldrin than its susceptible counterpart. This tendency does not necessarily indicate that absorption by the soluble components of the resistant strains is significantly higher than that of the susceptible strain. The radioactivity recovered from the resistant solvent phases was also high; the interstrain difference could be caused by the difference in the true substrate concentration, which is secondarily controlled by the rate of absorption by other particulate fractions.

Previously, the most conspicuous interstrain difference was in the crude-nucleus fraction (Table 1). The rate of absorption of dieldrin by this fraction was investigated at various substrate concentrations by first incubating C^{14}-dieldrin for 1 hour with rewashed nucleus fractions, and then collecting and rewashing the fractions twice with fresh sucrose solution (Fig. 1). For each strain there is a saturation plateau that deviates from the theoretical absorption line at low concentrations (the components causing the plateau and the linear absorption are designated α and β, respectively); the resistant (London) nucleus shows a low plateau, α. The interstrain difference in terms of t-value at $10^{-8}M$, for instance, was 3.21; it is highly significant at the 95-percent confidence level. The absorption constant for the susceptible component α, as judged by the median-saturation substrate concentration at equilibrium, was $9.1 \times 10^{-7}M$; that for the resistant component was $1.25 \times 10^{-6}M$.

A similar experiment with different incubation periods, at a dieldrin concentration of $1 \times 10^{-6}M$ (Fig. 2), indicated that the susceptible component

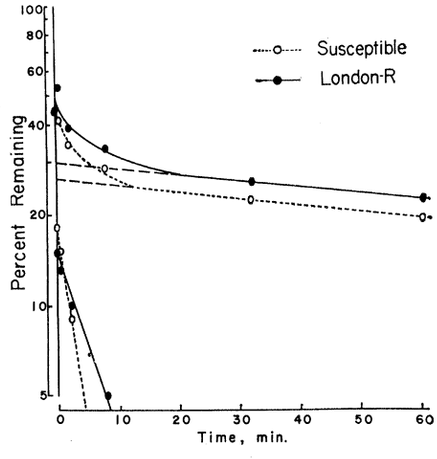

Fig. 2. Effect of time on the rate of binding of C^{14}-dieldrin with the nerve components of susceptible and resistant roaches. Percent Remaining (calculated) is the free dieldrin remaining at the end of the reaction as a percentage of the total dieldrin added initially. The fast phase of the main curve (bottom left) was obtained by subtraction of the straight line extrapolated to zero time.

(possibly α) had higher binding speed (bimolecular constant, 2.9×10^5 liter mole^{-1} min^{-1}) than the resistant counterpart (bimolecular constant, 1.4×10^5 liter mole^{-1} min^{-1}). The constants for the slow-binding components (possibly β) for each strain, on the other hand, scarcely differ from each other, the values being 4.9 and 5.3×10^3 liter mole^{-1} min^{-1} for the susceptible and resistant components, respectively.

It may be premature to state that all these tendencies of dieldrin-resistant strains, to have less binding capacity of nerve components with dieldrin, are causally related to the mode of resistance in these strains, for any insect colonies from different geographical locations can be expected to have a number of biochemical variations. To show that the two phenomena, dieldrin-resistance and binding of dieldrin with nerve components, are related to each other, genetic analyses [such as those employed to correlate low aliesterase activity with organophosphate resistance (*10*)] or reasonable biochemical evidence must be offered. As yet, no evidence indicates that dieldrin forms a charge-transfer complex with the nerve components of the German cockroach; dieldrin is unsuitable for ultraviolet-spectra analysis. The complex, unlike DDT complex, is inextractable with organic solvents: this fact indicates that the binding phenomenon, at least in part, involves a process of complex formation with nerve components other than simple lipids.

F. MATSUMURA
M. HAYASHI
Department of Entomology, University of Wisconsin, Madison

References and Notes

1. J. R. Busvine, *Nature* **174**, 783 (1954).
2. A. W. A. Brown, *Ann. Rev. Entomol.* **5**, 301 (1960).
3. D. G. Cockran, *Intern. Congr. Entomol. London Program* (1964), p. 34; G. Davidson and G. F. Mason, *Ann. Rev. Entomol.* **8**, 177 (1963); W. Klassen and A. W. A. Brown, *Can. J. Genet. Cytol.* **6**, 61 (1964).
4. F. P. W. Winteringham and A. Harrison, *Nature* **184**, 608 (1959); J. W. Ray, *ibid.* **197**, 1226 (1963).
5. E. S. Oonnithan and R. Miskus, *J. Econ. Entomol.* **57**, 425 (1964).
6. T. Yamasaki and T. Narahashi, *Botyu-Kagaku* **23**, 47 (1958).
7. ———, *ibid.*, p. 146.
8. R. D. O'Brien and F. Matsumura, *Science* **146**, 657 (1965).
9. A. W. A. Brown, *Bull. Entomol. Soc. Amer.* **10**, 138 (1964).
10. F. J. Oppenoorth, *Entomol. Exp. Appl.* **2**, 216 (1959).
11. Work supported by grants from the PHS (ES 00067-01) and the Research Committee, University of Wisconsin (64:408). Report approved for publication by the director of the Wisconsin Agricultural Experiment Station. The C^{14}-dieldrin was kindly provided by WHO.

11 April 1966

Triploid-Diploid Mosaic Chicken Embryo

Abstract. *Cytological analysis of an underdeveloped chicken embryo at 6 days of incubation revealed a triploid-diploid mosaic condition. Of the 30 metaphases observed, 19 were triploid and 11 diploid. The triploid cells were 3A-ZZZ and diploid cells 2A-ZZ, as determined for the six largest pairs of chromosomes.*

While the analysis of the normal chicken (*Gallus domesticus*) karyotype has advanced considerably, climaxed by Owen's report in 1965 (*1*), little has been reported with respect to chromosome number deviations. Newcomer *et al.* (*2*) demonstrated polyploid cells in the gonad of a sex-reversed female chicken. Ohno *et al.* (*3*) described an adult triploid chicken with a left ovotestis; the chromosome constitution was established to be 3A-ZZ. In a report on testicular chromosomes, Ford and Woollam (*4*) demonstrated a polyploid nucleus. This report of a triploid-diploid mosaic chicken embryo is a result of an effort to determine if the embryonic mortality among embryos from some matings may be attributed to aneuploidy or polyploidy.

Pedigree matings were made in a stock of Single Comb White Leghorn chickens mated with a male from a segregating population involving the Single Comb White Leghorn, Barred Plymouth Rock, and Cornish varieties of chickens. Embryos in a number of eggs from several females grew slowly, and a few appeared to be near death after 5 or 7 days of incubation. Owen's technique (*1*) was modified and employed on four of the very weak 5- to 7-day embryos. Two hours prior to killing, 0.1 ml of 0.05-percent colchicine was injected into the eggs near the developing embryo. The whole embryo was placed in a test tube and ground with a glass rod. The resultant macerated tissue was exposed to distilled water for 15 minutes. The cell suspension was centrifuged at 500 rev/min for 15 minutes, then the supernatant was decanted. Acetic-alcohol (1:3) fixative was added, and the pellet was resuspended slowly; fixation was for 30 minutes. After further centrifugation and decanting, the tissue was suspended in 45-percent acetic acid for 15 minutes. The cell suspension was filtered through cheese cloth to remove large clumps of tissue and other debris before air-dried preparations were made on a hot plate at 37° to 40°C. The slides were stained in aceto-orcein for 30 minutes, washed in 45-percent acetic acid, air-dried again, and mounted in Canada balsam. Observations were made by phase contrast microscopy.

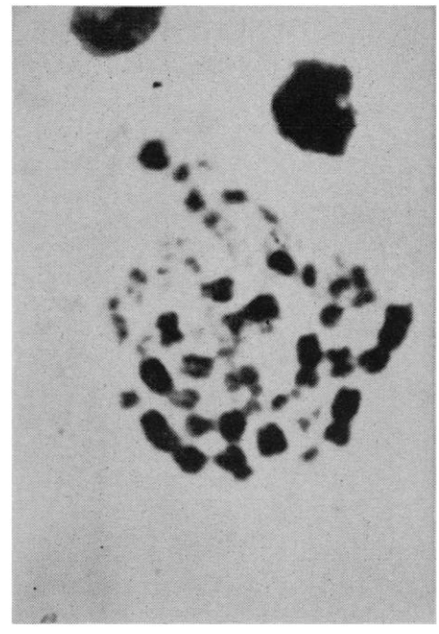

Fig. 1. Colchicinized metaphase from a chicken embryo containing a 3A-ZZZ chromosome constitution.

In one of the four embryos studied, triploid cells were observed. In this abnormal embryo, mitoses were rare, as was expected from the embryo's weak and underdeveloped condition.

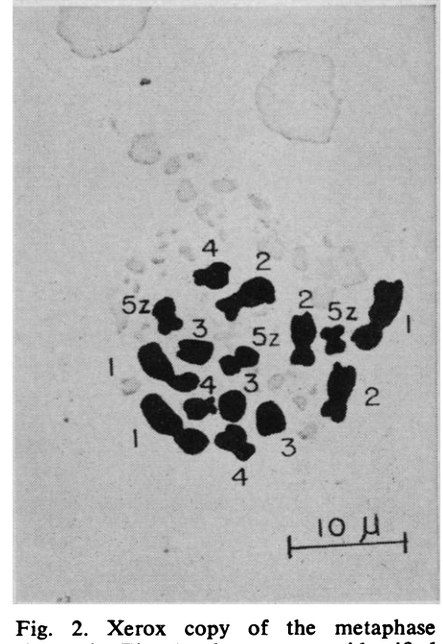

Fig. 2. Xerox copy of the metaphase shown in Fig. 1; chromosomes identified as numbers 1, 2, 3, 4, and 5 have been darkened with ink.