Aldrin Epoxidation:
At least from A to D!

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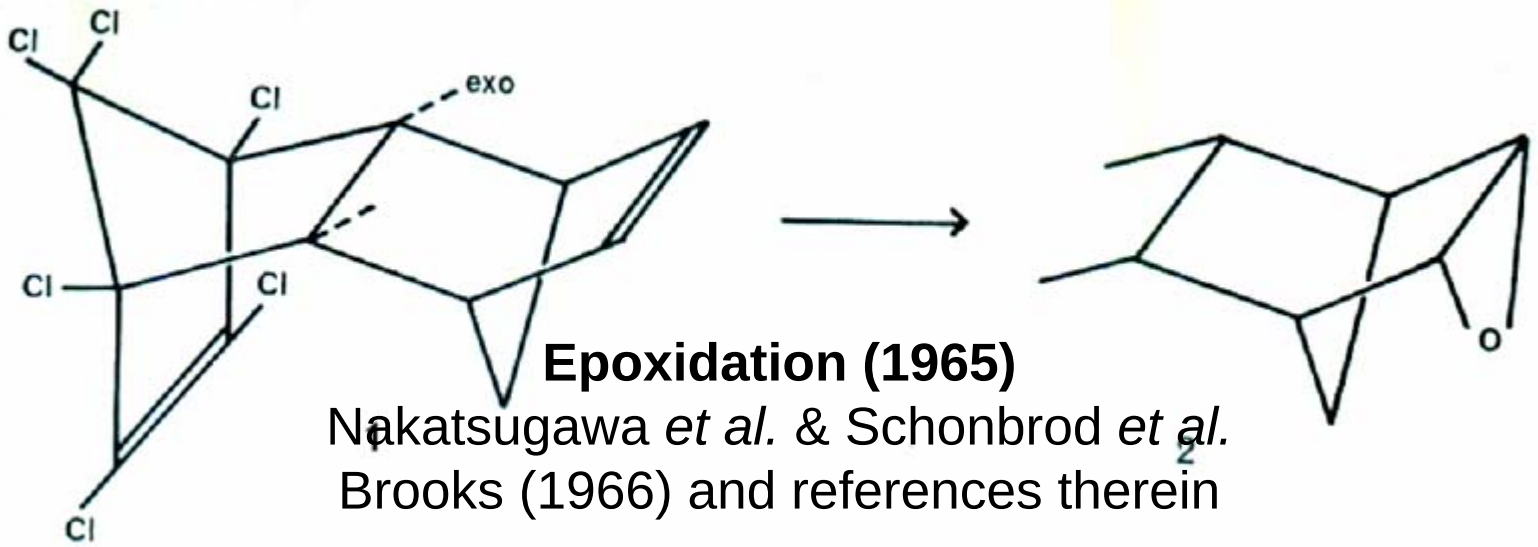
Aldrin

Biology

Chemistry

Dieldrin



Epoxidation (1965)

Nakatsugawa *et al.* & Schonbrod *et al.*

Brooks (1966) and references therein

Aldrin

- **Brooks, Agricultural Research Council, Pest Infestation Laboratory, Slough, UK**
- Lewis: housefly mcs, CO inhibition
- Ray: *in vivo* CO inhibition
- Wilkinson: MDPs, synergism

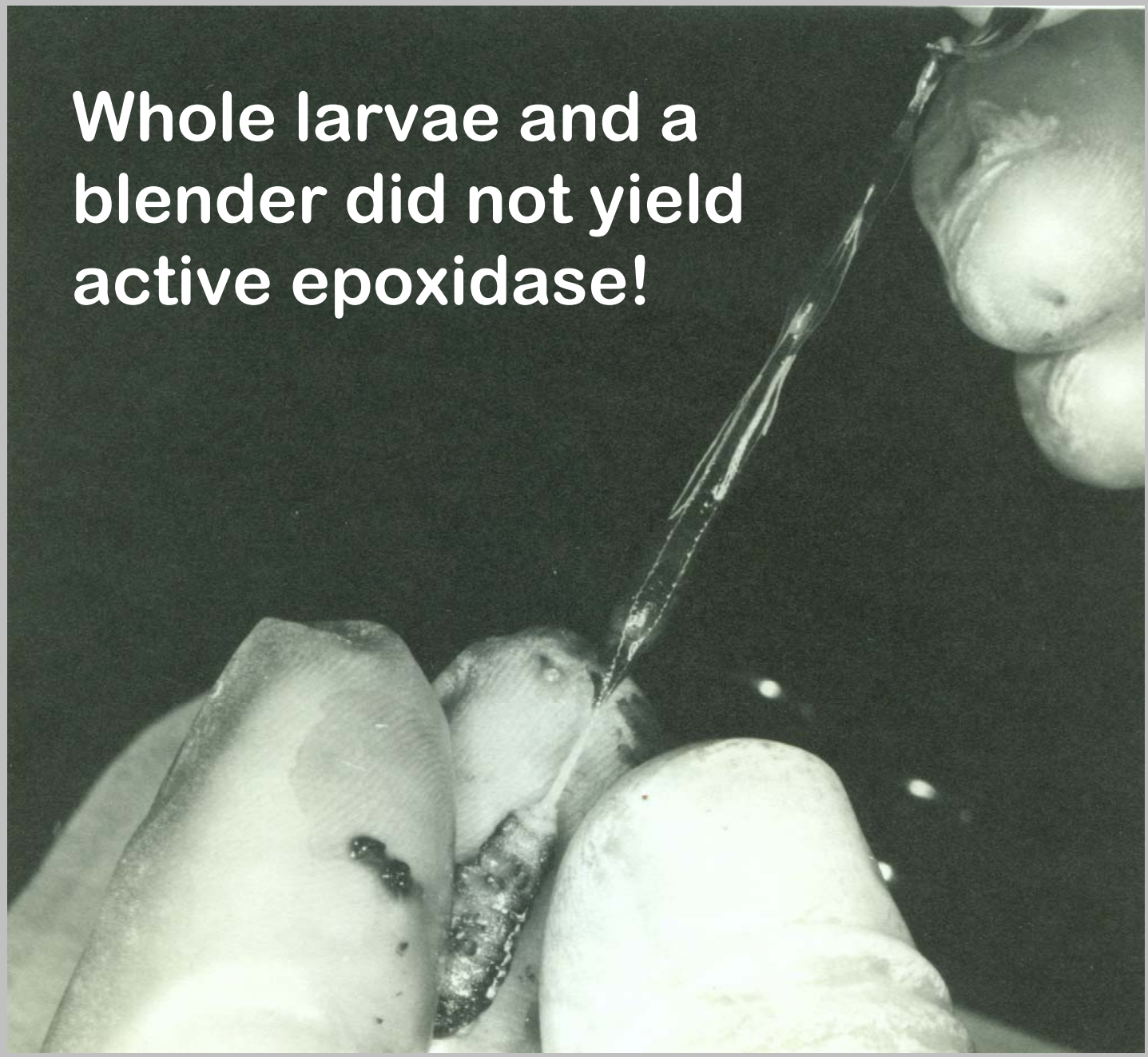
“Our ability to use to maximum advantage and in relative safety the vast number of drugs, pesticides and other lipophilic xenobiotics currently at our disposal is dependent to a large extent on our ability to establish their metabolic fate in living organisms.”

C. F. Wilkinson

Cornell University, 1979

Chemistry

- Southern armyworm *Prodenia eridania*
- Enzyme source: Microsomal mixed-function oxidases
- Substrates
  - Aldrin
  - Aniline
  - p-Chloro-N-methylaniline
- Cytochrome P-450


Whole larvae and a
blender did not yield
active epoxidase!

Tissue distribution of epoxidase activity in southern armyworm larvae

Tissue	nanomoles dieldrin/minute $x 10^3$		
	100 mg	larva	mg homogenate protein
Gut	**344**	**110**	**702**
Fat body	**55**	**23**	**42**
Malpighian tubules	**25**	**1**	**42**
Carcass	**4**	**5**	**7**
Head	**307**	**23**	**147**

Similar profile in 9 of 10 species (exception *Trichoplusia ni*)

Microsomal mixed-function oxidase activity of caterpillar gut tissue

Intracellular localization

Differential centrifugation

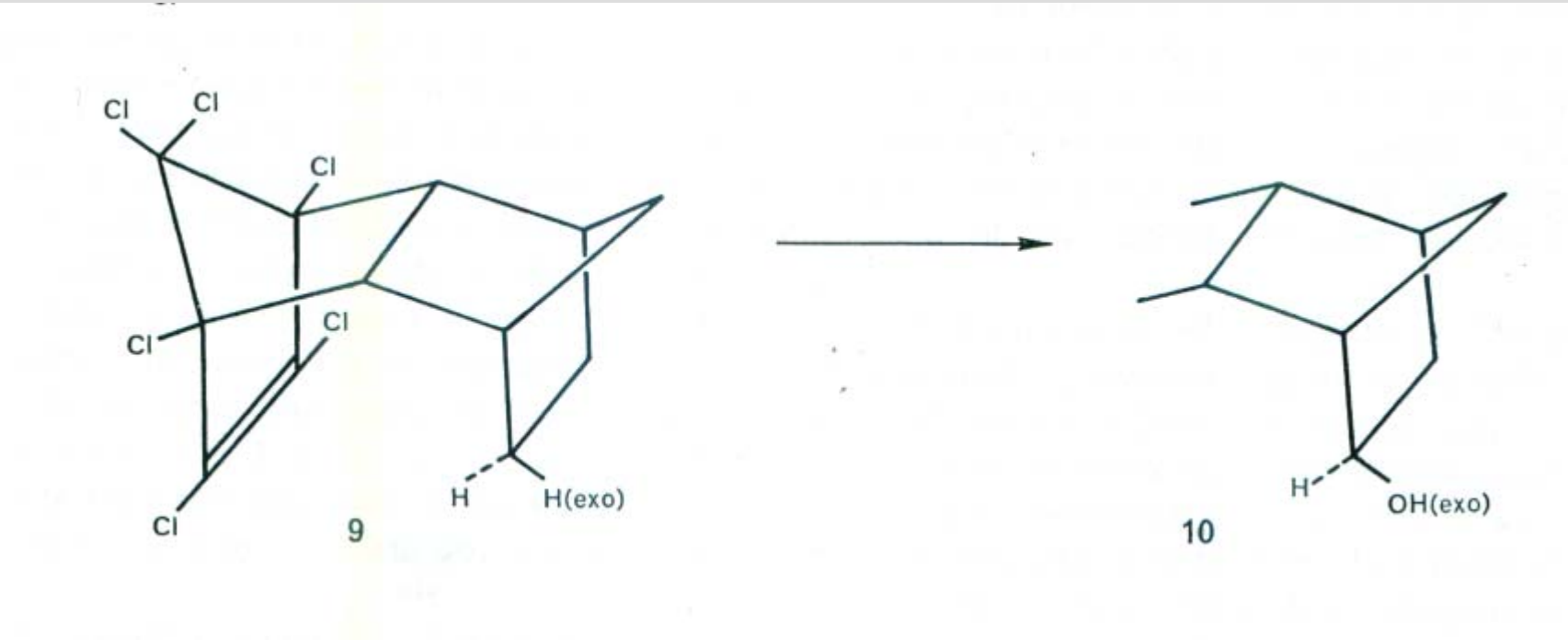
Discontinuous gradients

NADPH and O_2

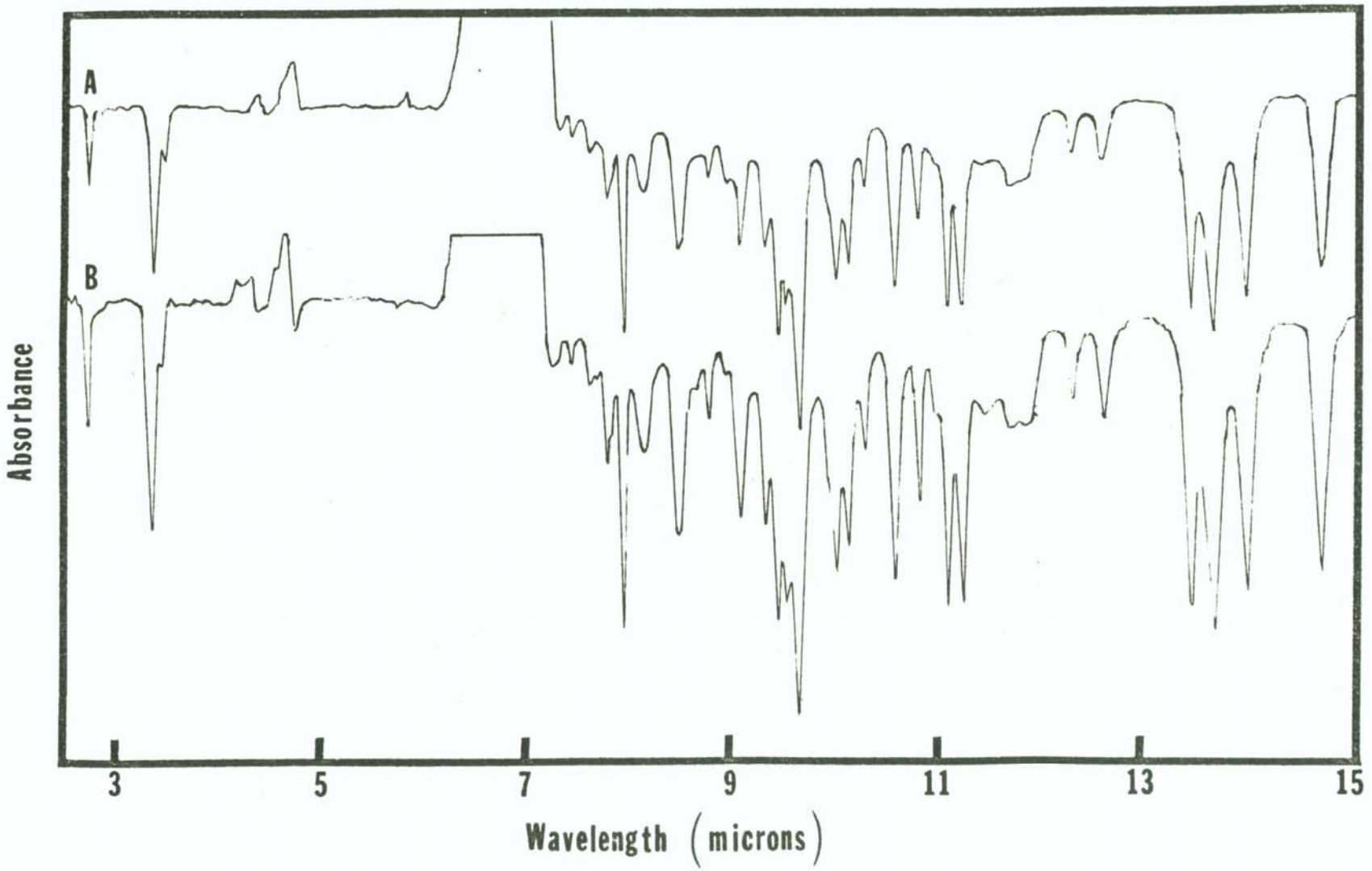
CO inhibition

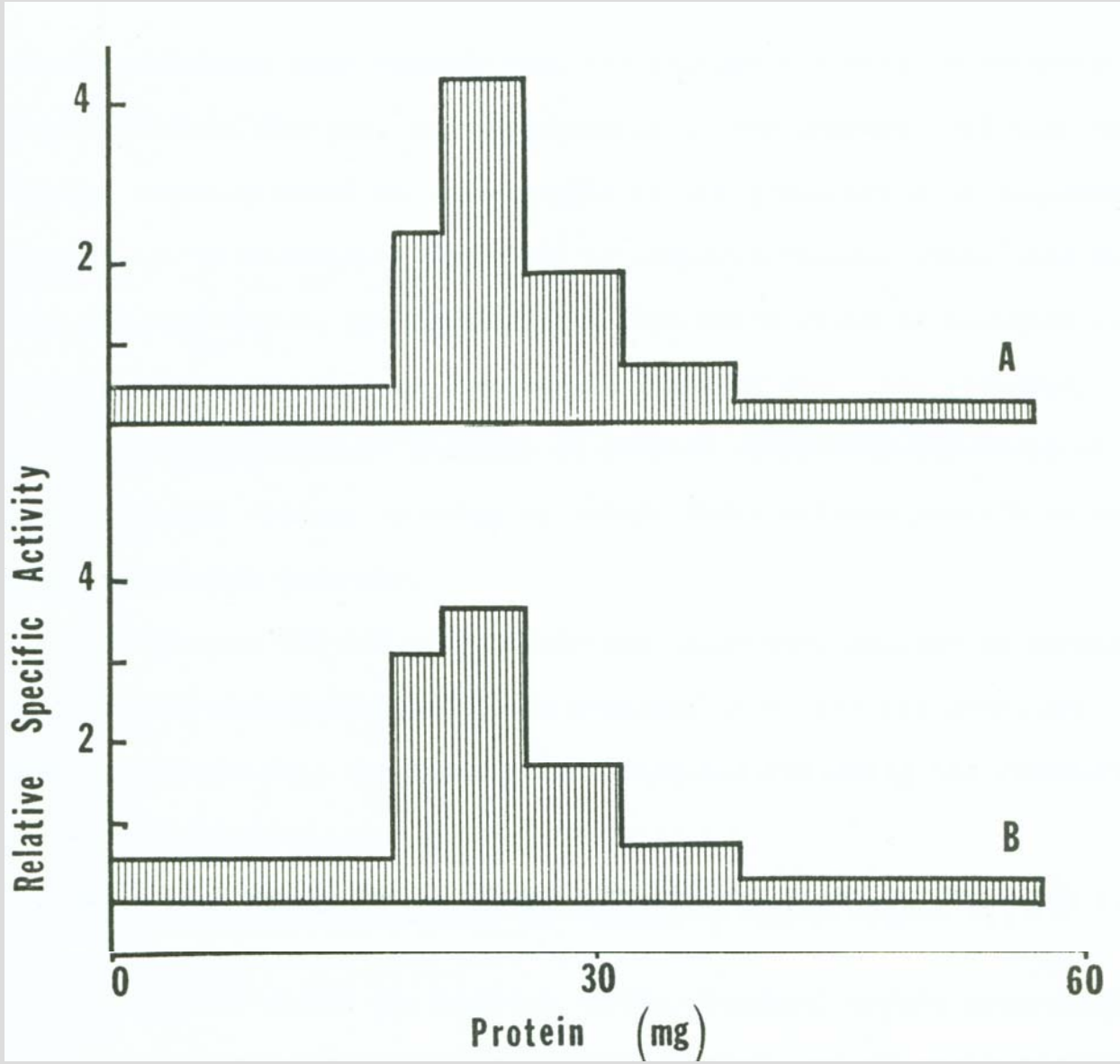
Substrates

- Aldrin — epoxidation
- Aniline — hydroxylation
- *p*-Cl-*N*-methylaniline — *N*-demethylation



Brooks, Progress in metabolic studies…,
Wrld Rev Pest Cntrl, 1966


A
B
Absorbance
3
5
7
9
11
13
15
Wavelength (microns)


4
2
A
4
2
B
Relative Specific Activity
0
30
60
Protein (mg)

Epoxidation and Hydroxylation

- Whole caterpillar mcs
- Gut (*minus* contents) *plus* ALD or DHI in buffered NADPH-generating system

- Foregut, **Midgut**, Hindgut
- Other species:
- **Snip, Snip, and Unzip!**

After the organism has been reduced to its constituent molecules, it is necessary to put it back together again….

E. Hodgson

Enzymatic Oxidations of Toxicants, 1968

Biology

1. Do ALD epoxidation and DHI hydroxylation represent the oxidative metabolic capability of living SAWs?
2. Does epoxidase activity in midgut signal function in metabolism of lipophilic chemicals in caterpillars?
3. New perspective….
    - adaptive advantage
    - CYP3A function in intestine

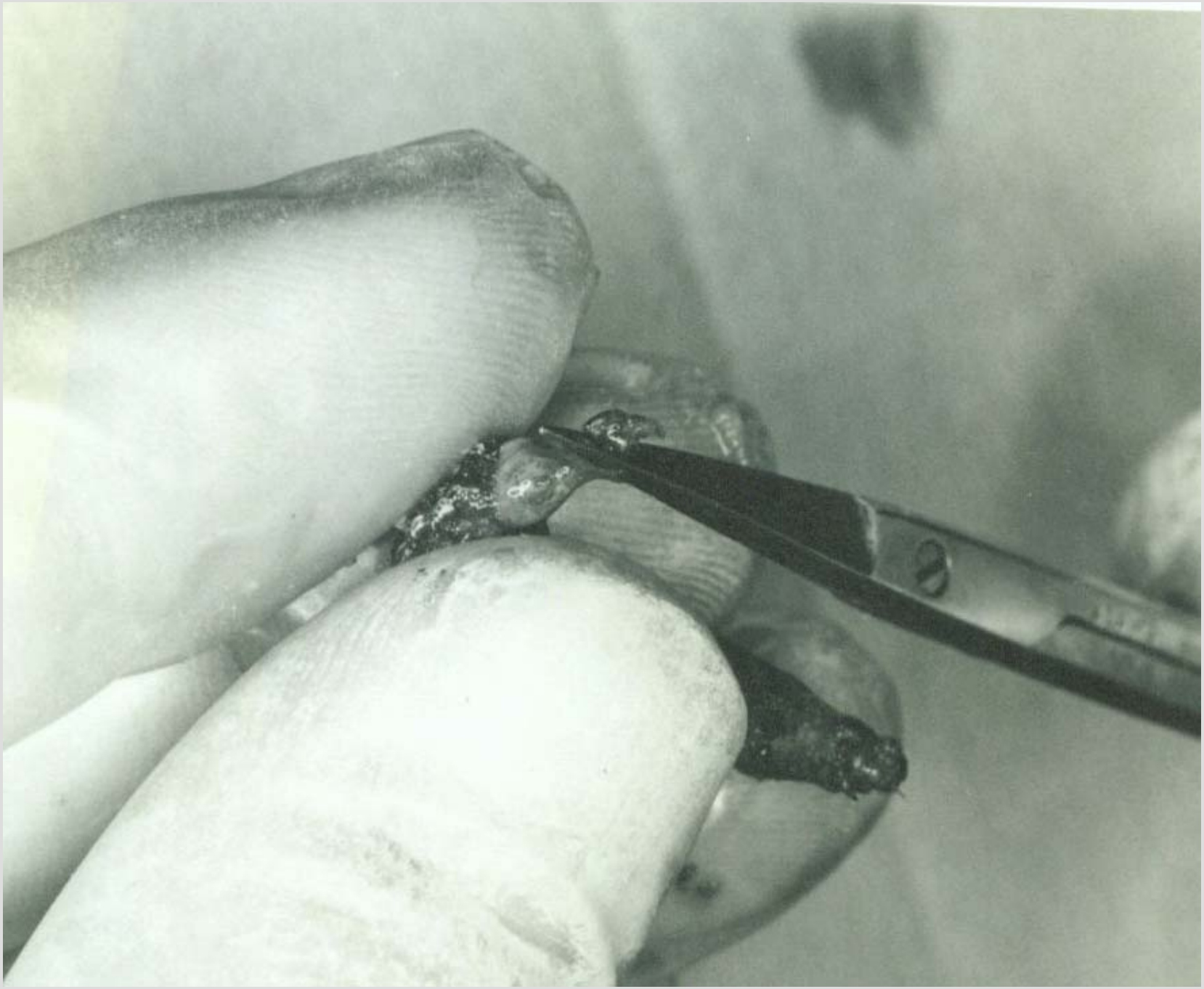
Cyclodiene toxicity in southern armyworms

Acute Toxicity	LD50 (mg/kg) 24h oral
Aldrin	14
Dieldrin	16
Isodrin	15
Dihydroisodrin	>450
Piperonyl butoxide	>500

Aldrin metabolism in southern armyworms

(Dieldrin / Dieldrin + Aldrin) x 100

Treatment	Hours 0	½	1	2	4	n
Aldrin 5 ug oral	**11**	**33**	**55**	**71**	**84**	**8**
plus oral pip butox (25 ug)	6	22	25	27	48	4
plus topical pip butox (25 ug)	-	29	61	70	81	2
pre- phenobar bital	18	37	61	76	83	2
pre- dihydrois odrin	-	-	62	-	-	2



Gut epoxidase activity of caterpillars and their host plant families:
Possible role in plant defences!

	Number of plant families		
Caterpillar (species)	monophagous **1**	oligophagous **2-10**	polyphagous **11 or more**
Saturniid (6)	0	4	2
Lasiocampid (2)	1	0	1
Geometrid (1)	0	1	0
Sphingid (1)	1	0	0
Notodontid (1)	1	0	0
Lymantrid (1)	0	0	1
Noctuid (17)	3	8	6
Arctiid (4)	0	2	2
Nymphalid (1)	1	0	0
Danaid (1)	1	0	0
Mean epoxidase pmole/mg-min, n=2>	**20.4**	**90.7**	**294.4**
n	8	15	12

Cabbage looper

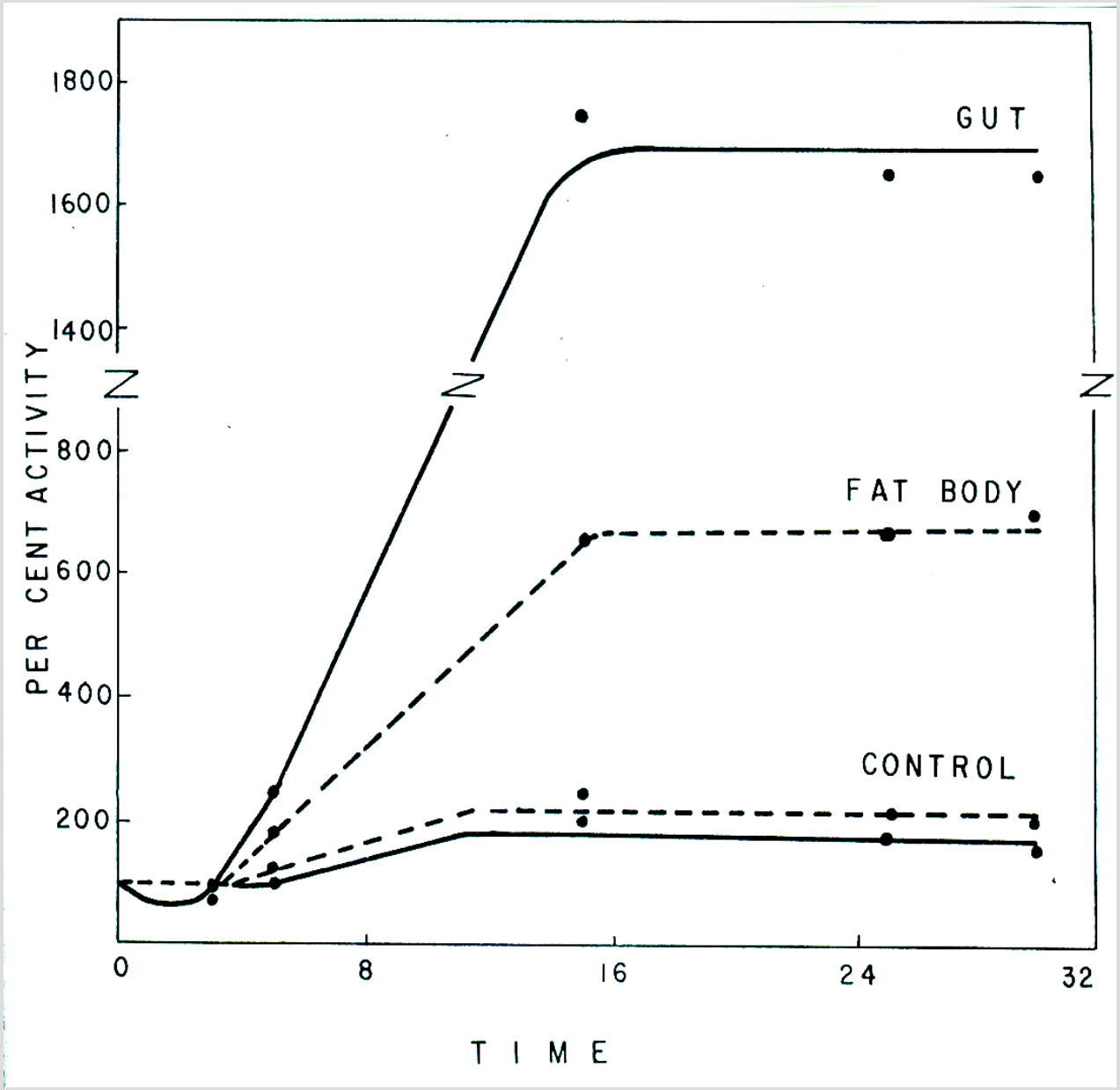
0.8 mg PipBtx

Topical DMSO:Ace 2:1

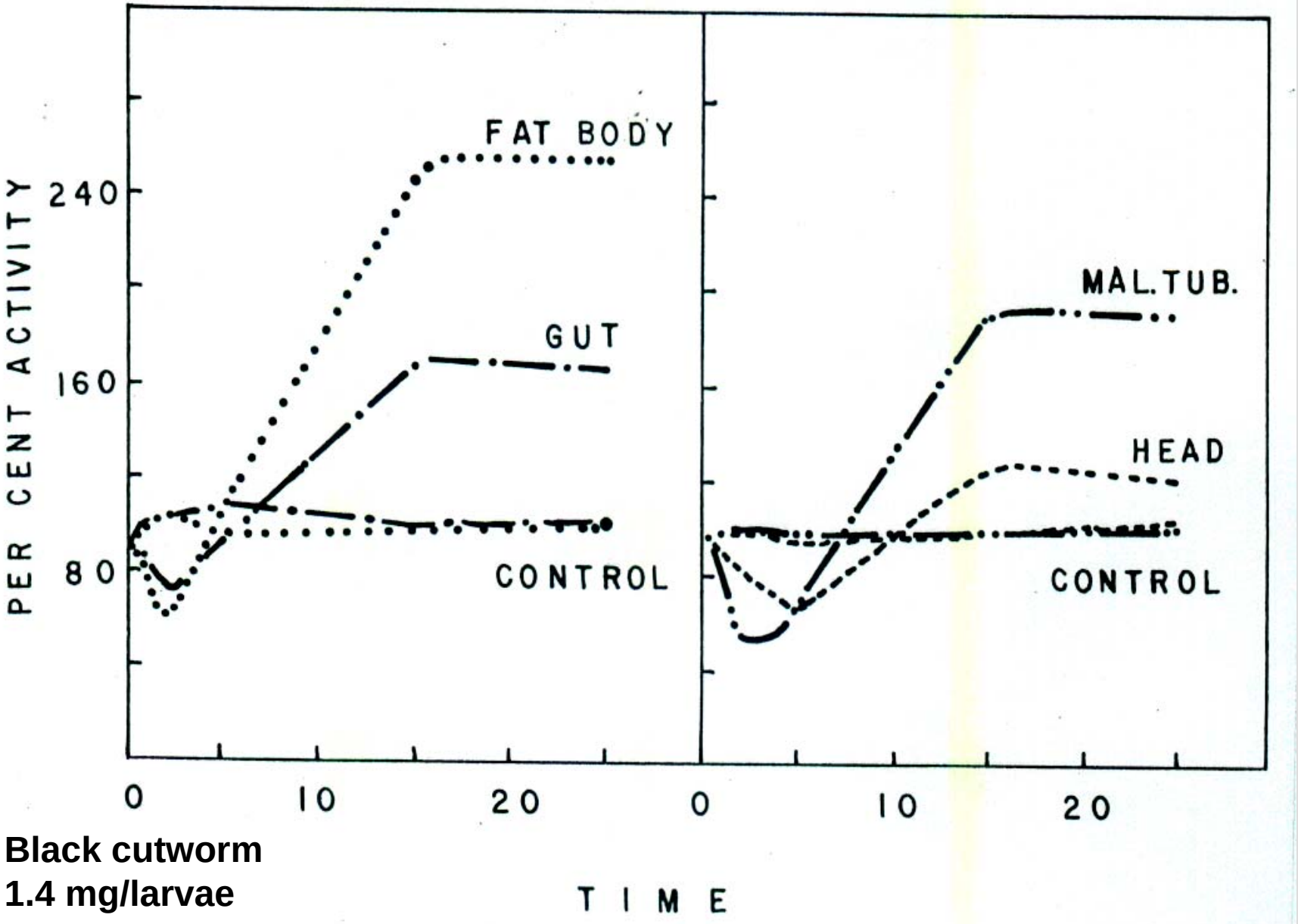
Homogenates

Time

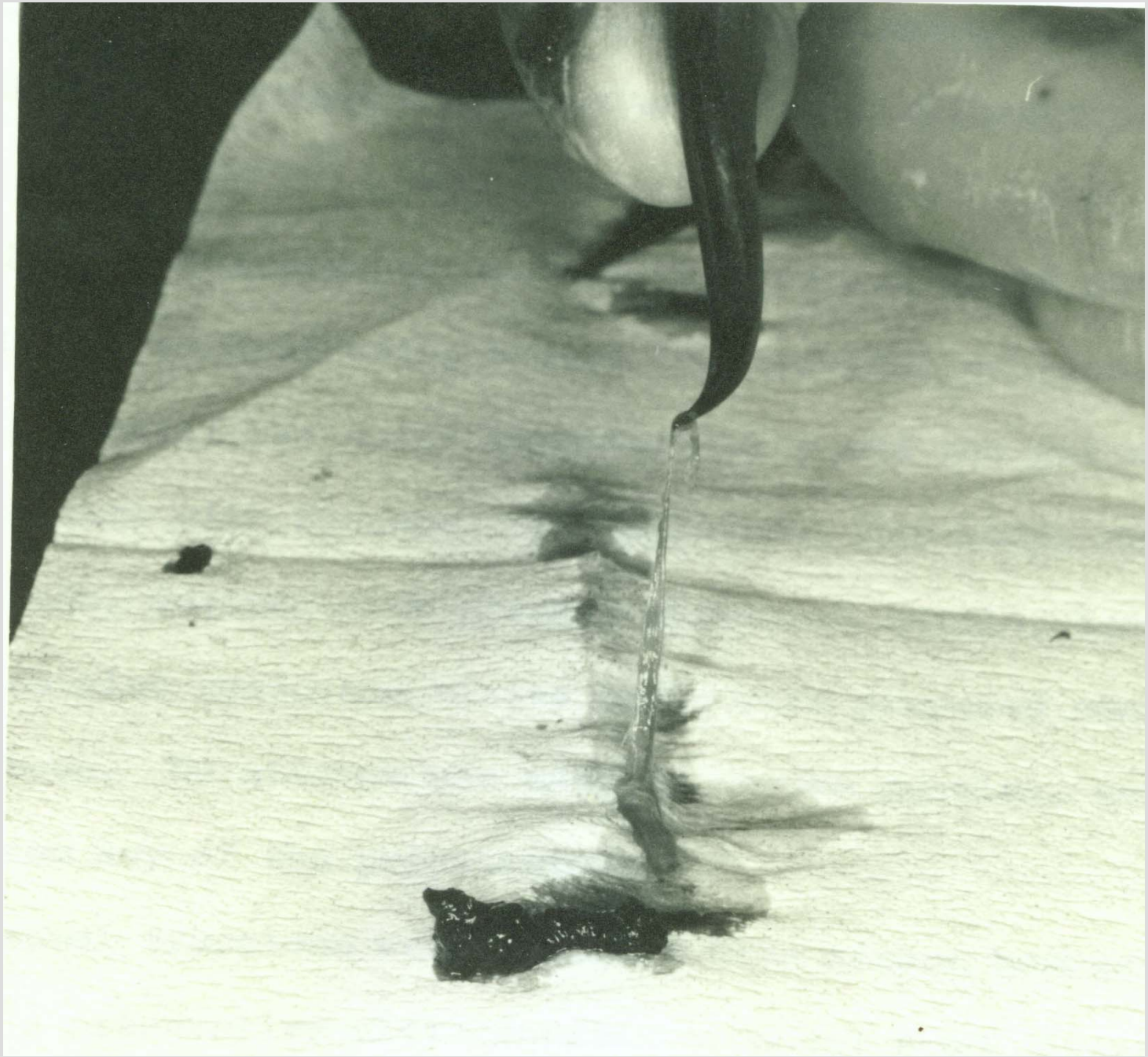
Dose

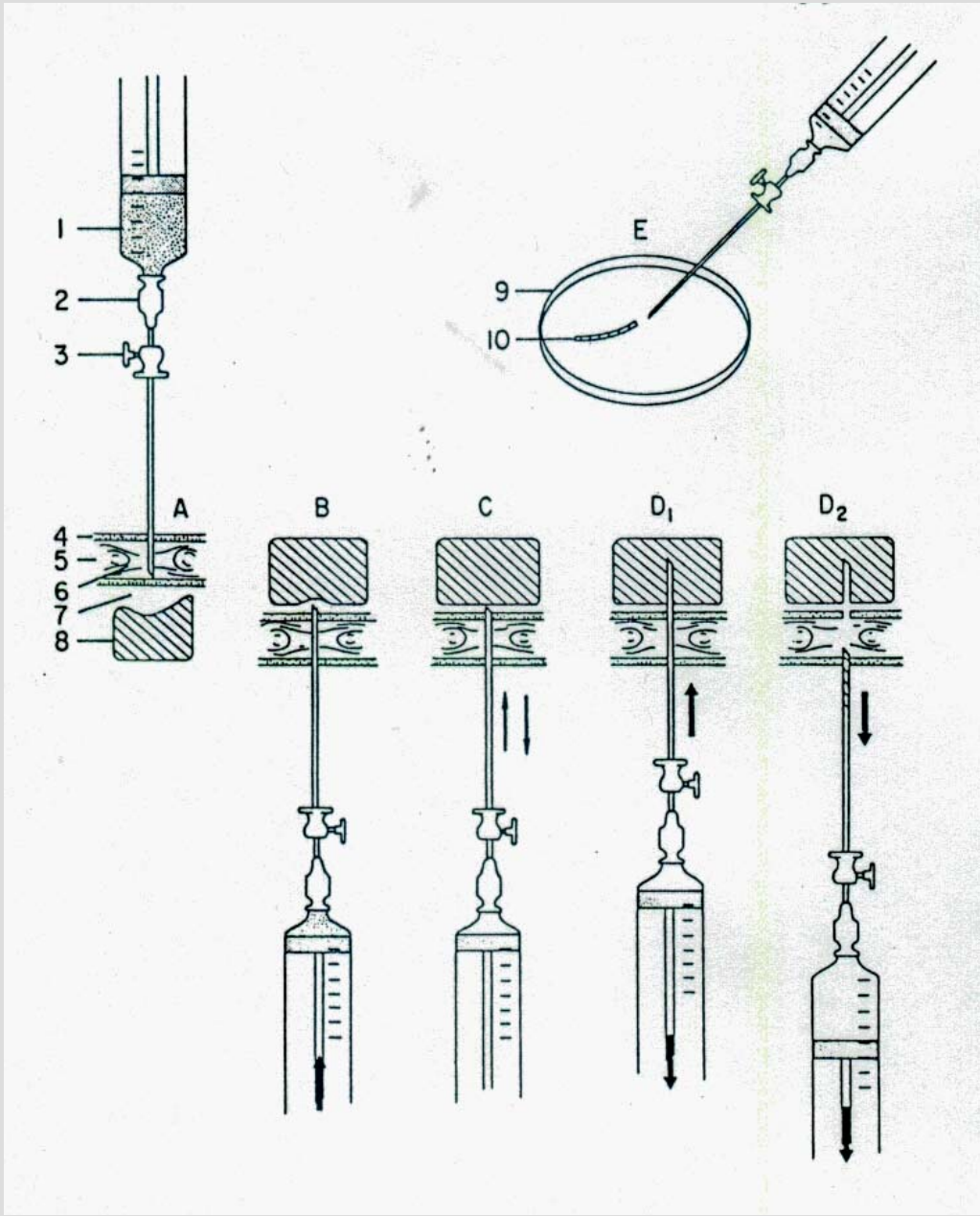


Piperonyl butoxide inhibition and induction of DHI homogenate hydroxylation



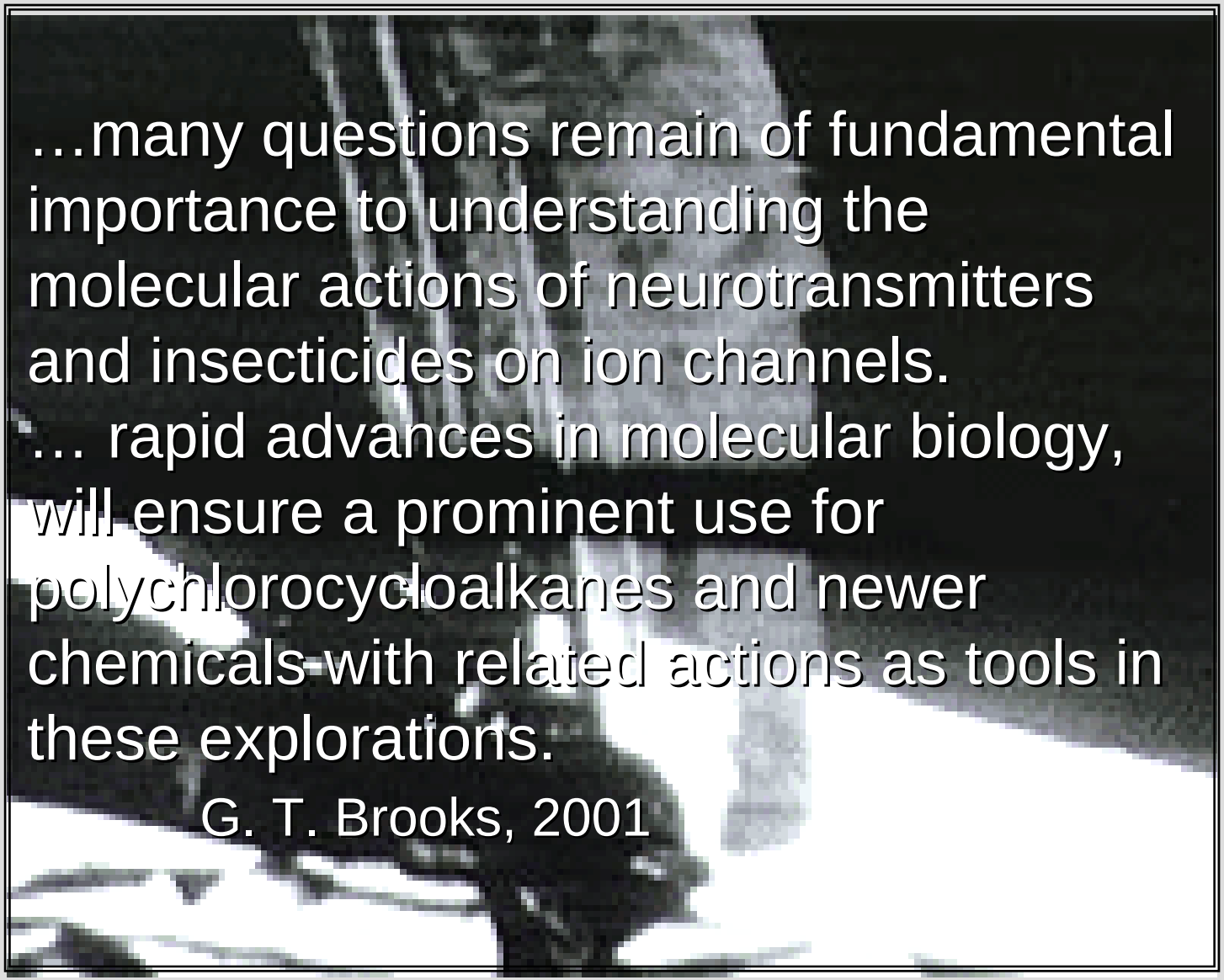
Black cutworm
1.4 mg/larvae




1
2
3
4
5
6
7
8
A
B
C
D1
D2
E
9
10

Function of intestinal CYP3A4

- cytochrome P450, family 3, subfamily A, polypeptide 4 [EC:1.14.14.1]
- First-pass metabolism in small intestine
- ALD epoxidase and P-450 association
- ALD epoxidation and DHI hydroxylation occur in small intestine



…many questions remain of fundamental importance to understanding the molecular actions of neurotransmitters and insecticides on ion channels.
… rapid advances in molecular biology, will ensure a prominent use for polychlorocycloalkanes and newer chemicals with related actions as tools in these explorations.

G. T. Brooks, 2001