# 17193. Effects of Certain Azo Dyes upon the Cockroach *Blattella germanica.**

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Very little is known about the effects of carcinogens upon insects. In the present study the cockroach *Blattella germanica* (L.) was fed crude or synthetic diets containing varying amounts of p-dimethylaminoazobenzene (DAB), m'-methyl-*p*-dimethylaminoazobenzene (m'DAB) or aminoazobenzene (AAB). The effects of the dyes upon growth rate, maturity, survival, ability to reproduce and the occurrence of internal lesions were determined. Newly-hatched nymphs from single egg sacs were divided into groups of 5-7 and kept in wire-screened test-tube cages[1] at a temperature of 27-32°C. Food and water were given *ad libitum* and were replaced weekly. The nymphs were weighed under $CO_2$ anaesthesia at 10, 20, and 30 days of age. The age at maturation, the ability to reproduce, and the fraction surviving were recorded. All surviving adults were eventually dissected and examined for abnormalities.

*Experimental.* In 3 preliminary experiments DAB was fed at 0.03, 0.06, 0.1 and 0.2%, and m'DAB was fed at 0.06 and 0.2% of a crude diet consisting of dog biscuits (Friskies) 94%, corn oil 4% and cholesterol 1 or 0.1%. Both the growth rate and reproductive performance of these insects remained essentially normal. The insects were dissected at ages varying from 54 to 151 days. At these times abnormalities of the digestive tract were seen in 37 of the 43 adults exposed to the dyes, while none of 50 insects fed only the crude diet were affected similarly. A common condition was the occurrence of small black flecks in the walls of the esophagus, crop, mid-gut and hind-gut and in the tissue of the fat body. A characteristic finding in insects fed 0.1% DAB was a degeneration of the Malpighian tubules, which were sticky and tended to form clumps or nodules. In some cases small vesicles were present at the proximal ends of the tubules. In Experiment 1, 3 of 5 roaches fed 0.1% DAB were affected, in Experiment 2, 4 of 5 and in Experiment 3, 0 of 5. The entire digestive tracts of these affected insects were removed, imbedded by the dioxan-paraffin method, cut serially into sections 6-10 microns thick and stained with hematoxylin, with or without eosin counterstain. Photomicrographs were taken of representative lesions (Fig. 1-3).

In a more detailed study, the potent carcinogens DAB and m'DAB were fed for long periods of time at a level of 0.2% in both crude and synthetic diets, and the non-carcinogen AAB was fed at 0.2% in a synthetic diet (Table II). One hundred and four newly-hatched nymphs from 4 egg sacs were distributed among 18 cages and fed the 6 diets shown in Table I. Growth rate, reproduction, and survival were measured, and the adults were dissected at ages varying from 112 to 173 days. Some of the $F_1$ nymphs from each group were kept on the same diets as their parents, and were killed for examination at 97-101 days of age. In addition to the experimental insects, a total of 127 adults previously fed a variety of crude and synthetic diets not containing azo dyes were dissected at ages varying from 53 to 143 days.

The growth rate, reproduction, and survival of the roaches were not greatly affected by the three azo dyes, although growth was retarded

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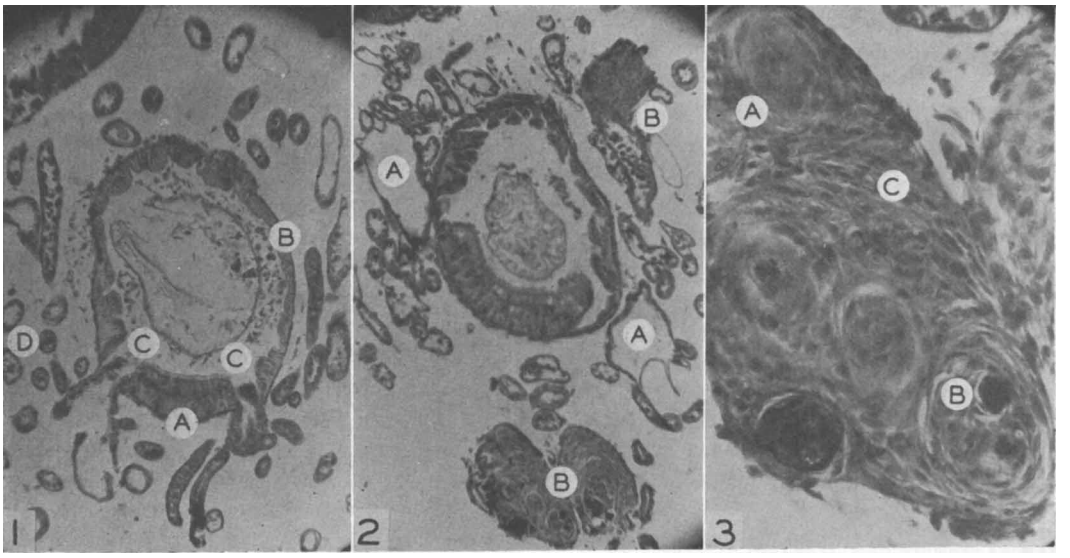
[1] Noland, J. L., Lilly, J. H., and Baumann, C. A., *Ann. Ent. Soc. Am.*, 1949, **42**, 63.




Cross-section of cockroach gut at the junction of mid-gut and hind-gut, showing lesions in the Malpighian tubules due to the feeding of azo dyes. A full description of this region as found in the normal cockroach is given by Miall and Denny.[2]

FIG. 1. Low Power. Cockroach previously fed 0.1% DAB for a short period of time. A—mid-gut. B—hind-gut. C—Origin of Malpighian tubules. D—Malpighian tubules in cross-section showing the beginning of degeneration.

FIG. 2. Low Power. Cockroach previously fed 0.1% DAB for a long time, showing the two characteristic types of lesions of the Malpighian tubules due to the azo dyes. A—Small vesicles formed by swelling of the tubules. B—Nodules formed by the fusion of adjacent tubules.

FIG. 3. High Power. Detail of a nodule shown in Fig. 2. A—Outlines of Malpighian tubules within nodule. B—Yellow granule of azo dye or its derivatives. C—Surrounding layers of hemocytes.

slightly when m'DAB was fed in the crude diet (age at maturity increased from 55 to 59 days) or DAB was fed in the synthetic diet (age at maturity increased from 49 to 64 days) (Table I). No consistent effect upon either reproduction or survival could be detected. The characteristic abnormality in insects fed the azo dyes in these experiments was the occurrence of large vesicles at one or more of the 6 points at which the Malpighian tubules arise from the intestine (see Fig. 1 and 2 for the location of these lesions). The vesicles ranged in size from one-fourth to 10 times the diameter of the mid-gut, and as many as 4 were observed in a single insect (Table II). The vesicles were usually thin-walled and filled with a light yellow fluid, but some were thick-walled and contained either a fluid or dark brown granules. In some individuals the proximal portions of adjacent Malpighian tubules were fused into nodules (Fig. 2 and 3). The extent of vesicle formation did not appear to be affected by the type of basal diet fed. Of the 3 dyes, DAB appeared to cause the most lesions, followed by m'DAB which was fairly active, and AAB which was slightly active (Table II).

*Discussion.* It is evident from these experiments that the cockroach has a much higher tolerance for the carcinogenic azo dyes than has the rat or mouse. 0.06% DAB or m'DAB in the diet seriously retards growth and prevents reproduction in the rat[5,6] while more than three times this level (0.2%) of either

[2] Miall, L. C., and Denny, A., The Cockroach, London, 1886, 123.

[3] Noland, J. L., Lilly, J. H., and Baumann, C. A., *Ann. Ent. Soc. Am.*, in press, II.

[4] Noland, J. L., and Baumann, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 198.

[5] Miller, J. A., Miner, D. L., Rusch, H. P., and Baumann, C. A., *Cancer Res.*, 1941, **1**, 699.

[6] Giese, J. E., Miller, J. A., and Baumann, C. A., *Cancer Res.*, 1946, **6**, 337.

TABLE I.
Growth Rate, Reproduction and Survival of Cockroaches Fed Various Azo Dyes.

| Basal* | Diet supplement† | Wt at 30 days, mg | Age at maturity, days | Egg-sacs produced | Avg No. per egg-sac | Survival (fract.) |
|---|---|---|---|---|---|---|
| Crude | None | 29 | 55 | 3 | 32 | 14/17 |
| " | DAB | 25 | 55 | 9 | 25 | 14/18 |
| Synt. | " | 31 | 64 | 1 | 29 | 11/18 |
| Crude | m'DAB | 20 | 59 | 5 | 28 | 9/12 |
| Synt. | " | 25 | 57 | 7 | 20 | 15/17 |
| " | AAB | 36 | 49 | 9 | 29 | 14/17 |

* The crude basal diet consists of "Friskies" dog biscuits 95% and corn oil 5%. The synthetic basal diet is "synthetic diet V."[3,4]

† All supplements were fed at 0.2% of the diet. DAB = *p*-dimethylaminoazobenzene; m'DAB = m'-methyl-*p*-dimethylaminoazobenzene; AAB = aminobenzene.

TABLE II.
Occurrence of Vesicles in the Malpighian Tubules of Cockroaches Fed Various Azo Dyes.

| Diet* | | Roaches dissected | | | Incidence of vesicles | | |
|---|---|---|---|---|---|---|---|
| Basal | Supplement | Age, days | Sex M | Sex F | % incidence | Avg No. per roach | Avg diam.† |
| | | First generation roaches. | | | | | |
| Crude | None | 112-123 | 10 | 6 | 0 | 0 | 0 |
| " | DAB | 112-123 | 7 | 6 | 58 | 2.0 | 2.4 |
| Synt. | " | 112-123 | 6 | 3 | 89 | 2.1 | 2.5 |
| Crude | m'DAB | 112-123 | 6 | 4 | 30 | 1.3 | 1.2 |
| Synt. | " | 112-123 | 7 | 6 | 75 | 1.3 | 0.6 |
| " | AAB | 112-173 | 4 | 3 | 29 | 1.3 | 0.6 |
| | | Second generation roaches. | | | | | |
| Crude | None | 97 | 0 | 10 | 0 | 0 | 0 |
| " | DAB | 97 | 5 | 10 | 100 | 1.7 | 2.5 |
| Synt. | " | 97 | 4 | 11 | 87 | 1.7 | 1.2 |
| Crude | m'DAB | 97 | 7 | 8 | 53 | 1.8 | 0.7 |
| Synt. | " | 97 | 3 | 10 | 46 | 1.7 | 0.5 |
| " | AAB | 101 | 7 | 6 | 69 | 1.6 | 0.6 |
| | | Unselected roaches. | | | | | |
| Crude | None | 90 | 25 | 25 | 0 | 0 | 0 |
| Fed 19 different synthetic diets | | 53-143 | 38 | 39 | 0 | 0 | 0 |

* For composition of diets, see footnote to Table I.

† Expressed as the ratio of the diameter of the vesicle to that of the mid-intestine, which is normally 0.75 mm in diameter.

compound had little or no effect upon the growth rate of the roach. Another difference is the relative order of activity of the 3 compounds fed. For the production of liver tumors in rats, m'DAB is much more active than DAB, and AAB is quite inactive[6,7] while for the production of Malpighian tubule lesions in the roach, DAB appeared to be more active than m'DAB, and AAB was also somewhat active. It is of interest that in the rat, tumors due to azo dyes are seldom found outside the liver[8] while in the roach, nodules or vesicles appeared only in the proximal region of the Malpighian tubules. Whether these latter lesions are tumors is doubtful; certainly no proof of autonomous growth is at hand.

An indication of the probable mechanism by which the lesions are formed was obtained by consecutive examination of serial sections

[7] Miller, J. A., and Baumann, C. A., *Cancer Res.*, 1945, **5**, 227.

[8] Rusch, H. P., Baumann, C. A., Miller, J. A., and Kline, B. E., *A.A.A.S. Res. Conf. on Cancer*, 1945, 267.

of affected tissues. Apparently the azo dyes are absorbed from the gut into the hemolymph and are then taken up by the Malpighian tubules. If the tubules cannot excrete the dyes or their degradation products into the hind-gut, as is ordinarily done with other excretory substances,[9] the dyes or their derivatives will accumulate in the proximal portion of the tubules. Occlusion of the open ends of the tubules could lead to their swelling into vesicles (Fig. 2) as the absorption of fluid by the tubules continues. If the ends remain open, but the dyes stay within the tubules, local effects of the dye could cause the tubules to degenerate, become sticky and adhere to adjacent tubules.. Hemocytes then accumulate around the affected areas to form nodules (Fig. 2 and 3). Wigglesworth[10] cites similar cases in which the hemocytes of insects encapsulated internal parasites, or walled off areas of infection or irritation. An alternative explanation of nodule formation would be that the nodules are tumors which were formed by the action of the azo dyes upon the Malpighian tubules. However, there appears to be no evidence at present which would lend support to this view.

*Summary.* The azo dyes *p*-dimethylaminoazobenzene (DAB), m'-methyl-*p*-dimethylaminoazobenzene (m'DAB) and aminoazobenzene (AAB) were fed to the roach *Blattella germanica* (L) at varying levels in both crude and synthetic diets. A dietary level of 0.2% of the dyes had little or no effect upon growth rate, survival or reproduction of the insects. However, characteristic lesions at the proximal ends of the Malpighian tubules occurred in roaches fed the dyes. DAB was the most active, m'DAB was next, and AAB was least active of the compounds fed.

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## 17194. Relation of Pantothenic Acid to White Blood Cell Response of Rats Following Stress.*

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Recent work has emphasized the role of the adrenal cortex in controlling the number of lymphocytes in the circulating blood following various forms of stress. Corticosterone produced a lymphopenia when injected into either adrenalectomized or intact mice.[1] The injection of the adrenocorticotropic hormone (ACTH) of the pituitary also produced a lymphopenia, provided the adrenals were present.[1,2] A deficiency of pantothenic acid causes lipid depletion and necrosis of the adrenal cortex.[3-6] Because of the importance of pantothenic acid to the physiological integrity of the adrenal cortex and in view of the influence of the adrenal cortex on the number of white blood cells, experiments have been done to determine the effect of diets adequate and deficient in pantothenic acid on the response of the white cells of rats subjected to stress.

*Experimental.* Young male rats of the Long-Evans strain, bred in the laboratory,

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3 Morgan, A. F., and Simms, H. D., *Science*, 1939, **89**, 565.

4 Daft, F. S., and Sebrell, W. H., *U. S. Public Health Reports*, 1939, **54**, 2247.

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