Table V. Digestibility Coefficients (%) on Crude Protein of Complete Ration

(Grain concentrate, silage, and hay)

Heifer No.	Hemicellulose Extract Ration Crude Protein	Heifer	Control Ration Crude Protein
992	67.8	994	54.3
993	67.1	995	67.0
996	60.2	998	72.5
997	53.3	999	55.9
Av. per group	62.1		62.4

Hay and silage were kept at a minimum in order that the heifer would receive most of the protein equivalent content from the grain. The total protein digestibility coefficient for the total ration as determined at 50% ammoniated material in the ration fed to this animal was 60.6%, which compares favorably with the other animals on trial (Table III).

Ammonia nitrogen may be absorbed and excreted in the urine; however, other experimentation (*8*), using the same by-product with Holstein lactating cows, has shown no increase in ammonia nitrogen (Van Slyke–Cullen aeration method) or total protein in the urine when compared to the control ration not containing the ammoniated by-product (hemicellulose extract, 30.0 mg., and control, 28.0 mg. of ammonia per liter of urine, respectively).

Feed refusals, digestive disturbances, fecal abnormalities, and general ill effects were negligible on both rations during the 90-day feeding trial. Hair coats and general appearances of the heifers fed the hemicellulose extract compared favorably to the heifers receiving the control ration.

Literature Cited

(1) Bartlett, S., and Cotton, A. G., *J. Dairy Research*, **9,** 263–72 (1938).
(2) Briggs, H. M., Gallup, W. D., Darlow, A. E., Stephens, D. F., and Kinney, C., *J. Animal Sci.*, **6,** 445–60 (1945).
(3) Culberston, C. C., Shearer, P. S., Hammond, W. E., and Moore, S., Iowa State College, Agr. Ext. Service, **A.H. 176** (1950).
(4) Harris, L. D., and Mitchell, H. H., *J. Nutrition*, **22,** 183–96 (1941).
(5) Hart, E. B., Bohstedt, G., Deobald, H. J., and Wegner, M. I., *J. Dairy Sci.*, **22,** 785–98 (1939).
(6) Kane, E. A., Jacobson, W. C., and Moore, L. A., *J. Nutrition*, **41,** 583–96 (1950).
(7) Knodt, C. B., Williams, J. B., and Brumbaugh, J., *J. Animal Sci.*, **9,** 661–2 (1950).
(8) Magruder, N. D., Knodt, C. B., and Williams, P. S., unpublished data.
(9) Miller, H. C., *J. Dairy Sci.*, **27,** 225–41 (1944).
(10) Morrison, F. B., "Feeds and Feeding," 21st ed., Ithaca, N. Y., Morrison Publishing Co., 1948.
(11) Pearson, R. M., and Smith, J. A. B., *Biochem. J.*, **37,** 142–8 (1943).
(12) *Ibid.*, pp. 148–53.
(13) *Ibid.*, pp. 153–64.
(14) Rupel, I. W., Bohstedt, G., and Hart, E. B., *J. Dairy Sci.*, **26,** 647–63 (1943).
(15) Snedecor, G. W., "Statistical Methods," 4th ed., Ames Iowa, Iowa State College Press, 1948.
(16) Tillman, A. D., and Kidwell, J. F., *J. Animal Sci.*, **10,** 934–8 (1951).
(17) Wegner, M. I., Booth, A. N., Bohstedt, G., and Hart, E. B., *J. Dairy Sci.*, **24,** 835–44 (1941).
(18) Willett, E. L., Henke, L. A., and Maruyame, C., *Ibid.*, **29,** 629–37 (1946).
(19) Work, S. H., and Henke, L. A., *Proc. Am. Soc. Animal Production*, **1938,** 404–6.

Received for review August 14, 1953. Accepted September 11, 1953. Research supported in part by a grant from the Commercial Solvents Corp., New York, N. Y. Data from a thesis to be submitted to the Graduate School of the Pennsylvania State College in partial fulfillment of the requirements for the degree of doctor of philosophy by the senior author. Authorized for publication as paper No. 1691 in Journal Series of the Pennsylvania Agricultural Experiment Station.

Schradan Metabolism Related to Tissue Enzyme System

SCHRADAN

Metabolism of Octamethylpyrophosphoramide by Insects

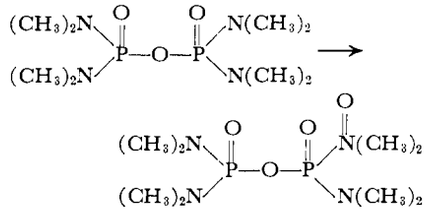
R. D. O'BRIEN, Department of Chemistry, University of Western Ontario, London, Canada
E. Y. SPENCER, Science Service Laboratory, Department of Agriculture, London, Canada

SCHRADAN IS OF CONSIDERABLE INTEREST as one of the first organic systemic insecticides. Besides being systemic, it has two other important properties. First, it is selective, killing only sap-sucking insects, most of which belong to the order Hemiptera. This was first observed in the field (*25, 26*) and has been confirmed by more direct methods (Table I). Secondly, it appears to kill by inhibiting the cholinesterase of insect nerves, judging by the symptoms shown by poisoned insects and by the observation of Duspiva (*7*) that poisoned aphids had their cholinesterase strongly inhibited; yet it is in vitro a very weak anticholinesterase, against erythrocyte cholinesterase (*10*), serum cholinesterase (*30*), and bee brain cholinesterase (*27*). Presumably, a conversion occurs in the plant or the insect (or both) from a weak to a strong anticholinesterase.

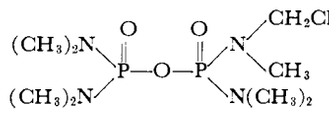
Following their observation that mammals are susceptible to schradan poisoning, Dubois *et al.* (*5*) showed that mammalian liver slices could accomplish such a conversion. Cheng (*3*) showed that this mamalian conversion occurred only in the liver, by demonstrating that schradan is nontoxic to the hepatectomized rat. Conversion can also be produced by plants to a small extent (*12*), by "animals, bacteria and insects" (*31*), and chemically by oxidation with permanganate (*2*) and by chlorination (*30*). Biological oxidation probably follows the

Susceptibility to schradan poisoning has been shown to be due to other factors than quantities ingested. Insect tissues from both susceptible and nonsusceptible species are able to convert schradan to a strong anticholinesterase. Differences in schradan-converting capacity between susceptible and nonsusceptible insects are quantitative; on this basis a theory of the mode of action of schradan has been suggested. The conversion has been shown to be a function of a tissue enzyme system. An attempt has been made to relate the occurrence of the converting system to the normal metabolic processes of insects and mammals.

scheme suggested by Hartley (*13*), the first step being the coordination of an oxygen with the nitrogen of a dimethylamido group:



This oxygen is electrophilic and renders the anhydride link unstable, the molecule becoming readily hydrolyzable and a strong anticholinesterase. The chlorinated product used in some of the work reported here is probably monochloroschradan:



along with very unstable di- and trichloro derivatives (half-lives at 25° C., 4 and 2 minutes, respectively). In this case the electrophilic chlorine renders the anhydride link unstable. Monochloroschradan inhibits cholinesterase by 50% at about $10^{-7}M$ whereas schradan itself inhibits 50% at about $10^{-2}M$ (*30*).

Selective Toxicity

To account for the selective toxicity of schradan, three hypotheses have been advanced:

That the cholinesterase of the susceptible insects is particularly sensitive. This was suggested by Martin (*19*) by analogy with the effect of diisopropyl *p*-nitrophenyl thiophosphate (analog of parathion), which is almost nontoxic to bees and is a poor inhibitor of bee brain cholinesterase in vitro, yet is very toxic to flies and a good inhibitor of fly brain cholinesterase in vitro (*27*).

That by their mode of feeding, sucking insects tend to take in high doses of translocated insecticide.

That only the susceptible insects contain an enzyme system that can convert schradan to a strong anticholinesterase. This was first suggested by Martin (*18*). Duspiva (*7*) came to the same conclusion after showing that the heads and thoraxes of (presumably) flame bugs (*Pyrrhocoris apterus*), a susceptible insect, had their cholinesterase strongly inhibited following incubation with schradan in vitro.

Materials and Methods

Source of cholinesterase was diluted human serum, made by centrifuging whole blood and diluting the serum to a convenient activity (b_{30} = 320 μl. of carbon dioxide) which usually required about 6 volumes of water. Acetylcholine bromide was the substrate. The standard treatment for investigating schradan conversion by tissues was as follows:

The tissue was dissected out under Ringer's solution, blotted, weighed (on a Roller-Smith balance), and transferred to a Warburg flask containing 1 ml. of diluted serum and 1 ml. of saline solution (0.9% sodium chloride, 0.025% potassium chloride, 0.018% calcium chloride). At zero time 0.25 ml. of 0.5% schradan was added, all constituents being now in the main compartment. The flask was attached to the manometer and shaken for 1 hour at 25° C., then removed and 0.3 ml. of 2.52% sodium bicarbonate was added to the main compartment and 0.2 ml. of 5% acetyl choline to the side arm. The flask was replaced on the manometer and put in the bath; 90 minutes after zero time, it was gassed with 95% nitrogen–5% carbon dioxide for 10 minutes. Temperature equilibration was allowed for 20 minutes, then the manometer was adjusted to zero and closed and readings were commenced. Substrate was tipped in 30 minutes later. Results were calculated by the procedure of Aldridge *et al.* (*1*). All determinations were replicated at least six times, or until a satisfactory standard error was obtained.

Schradan was prepared and purified as described in a previous paper (*30*). In the case of inhibitor studies, the inhibitor was added to the saline-serum before the tissue was added. All inhibitor concentrations given are final concentrations. Injections were made with a positive-action ratchet-operated injector designed by R. W. Fisher of this laboratory, and with an Agla micrometer syringe. Homogenization was made in a Potter-Elvejhem all-glass homogenizer.

The insects used were: American cockroach, *Periplaneta americana* (adults); yellow meal worm, *Tenebrio molitor* (larvae); mourning cloak, *Vanessa antiopa* (larvae); squash bug, *Anasa tristis* (adults); milkweed bug, *Oncopeltus fasciatus* (adults); blowfly, *Phormia regina* (adults).

Results given are means plus or minus standard errors of the means.

Results and Discussions

Toxicity of Schradan The toxicity of schradan toward the squash bug, milkweed bug, mourning cloak larvae, mealworm larvae, and blowfly was determined, with the results shown in Table I, which also includes the few data in the literature. The bugs (Hemiptera) and aphids (Homoptera) are seen to be intrinsically sensitive to schradan poisoning; their susceptibility as observed in the field is therefore not simply a function of their mode of feeding, such as the consumption of massive quantities or the selection of potent portions of the plant. It also follows that it is not necessary for the plant to act upon schradan in order to make it insecticidal.

The next point to consider was whether the differences in susceptibility to schradan of various insects were due to differences in the sensitivities of their cholinesterases. Duspiva (*7*) had shown that cholinesterase extracted from the nerve cord of (presumably) the flame bug (*Pyrrhocoris apterus*) was not inhibited by a commercial sample of schradan, "however high a concentration was used" (author's translation). A similar experiment was carried out with the squash bug and pure schradan. A brei of heads was used, equivalent to 30 heads per flask. No inhibition of their cholinesterase was shown by schradan at concentrations up to $2 \times 10^{-3}M$. Susceptibility is thus not associated with a sensitive cholinesterase. The authors therefore expected to find that one or more tissues of the susceptible insects would convert schradan to a powerful anticholinesterase and that this capacity would be lacking in the corresponding tissues of nonsusceptible insects. On the contrary, several tissues from all insects investigated contained a system capable of this conversion. Most of the work was done on the adult roach, a typical nonsusceptible insect. Fore-gut, mid-gut, hind-gut, fat body, nerve tissue, and

(to a very small extent) cuticle could convert schradan, but muscle was totally inactive (Table II).

Effectiveness Of Conversion The figures for relative effectiveness in the conversion require some explanation. For any given tissue, a poor correlation was found between tissue weight and conversion capacity; this was expected because the weight of the tissue included inactive material such as food debris in gut and fat in fat body. Therefore, in considering any given insect, results were expressed on a unit basis—e.g., comparing hind-gut and fore-gut, the activity of one whole fore-gut was compared with that of one whole hind-gut. In order to weight these results satisfactorily for the comparison of different insects, all results were expressed as "effectiveness per 100 mg. of body weight," the body weight used being the mean species weight. These results are included in Table II, where it will be seen that the standard errors of the means are small enough to permit this treatment. The mourning cloak caterpillars grew so rapidly that individual weights were used.

A linear relationship exists between the log concentration of an inhibitor and the resultant per cent inhibition; therefore, if tissue A converts enough schradan to give twice as great a cholinesterase inhibition as tissue B, A is producing much more than twice as much inhibitor as B. To convert the results appropriately, the slope of the plot of inhibitor concentration versus per cent inhibition of cholinesterase has to be known. Previous work on derivatives of schradan had shown that these slopes were parallel to each other and to that of schradan itself. For convenience, the curve for monochloroschradan was chosen and from it the concentration of monochloroschradan equivalent to any given inhibition was obtained; then from this figure the similarly converted effect due to schradan alone was subtracted and the result was multiplied (arbitrarily) by 10^7. [The following figures will serve to duplicate the curve: *pI* (negative log of molar concentration) = 6.0, inhibition = 41%; *pI* = 5.0, inhibition = 87%]. The result, divided by 1/100th of the average weight of the insect (in milligrams), is the figure reported as effectiveness per 100 mg. of body weight. Sometimes in studying small insects it was necessary to use tissues from more than one insect in each flask. Where, say, four tissues per flask were used, the "effectiveness" was obtained by dividing the results by 4. The validity of this process was checked by comparing the corrected results obtained for two roach fore-guts per flask with those obtained using one fore-gut per flask. Excellent agreement between the results was observed. Similarly, for the fat bodies of the larger insects the tissue from each insect was divided among four flasks, and the over-all result was obtained by adding the individual results.

The term "residuum" requires some explanation. In the mourning cloak caterpillar the fat body is rather diffuse and consequently difficult to dissect out completely. The remainder of the insect, after removing gut, nerve cord, and as much fat body as possible, was tested and termed residuum. Its activity is probably entirely due to its fat body content and the true fat body is probably the sum of the values given for fat body and for residuum. However, lacking evidence other than inferential, they have been reported separately.

The results in Table II show that in all insects studied, large quantities of schradan can be converted to a powerful anticholinesterase. Therefore any hypothesis which attempts to account for the differential toxicity of schradan must explain the failure of this powerful anticholinesterase to kill the nonsusceptible insects, and presumably this can be done only by suggesting that much converted schradan is either destroyed or fails to penetrate to the site of action. The results show two differences between the susceptible and nonsusceptible species; the former have fat bodies less effective and guts more effective than the latter in the activation of schradan. The differential toxicity may be due to the gut effect, the fat body effect, or to both together.

It seems improbable that the gut effect is important and the fat body effect is not, since schradan injected into the body cavity is in contact with the fat body to an extent at least as intimate as with the gut. There appears to be no spatial consideration to suggest that gut-converted schradan should have readier access to nervous tissue than that converted in the fat body. However, it is possible that the fat body effect is more important than the gut effect, since that portion of the schradan which was converted in the gut might be incapable of diffusing through the gut wall. Evidence that converted schradan does not diffuse through other membranes is given below. In the case of fat body this problem evidently does not arise, since the in vitro experiments use whole fat body under conditions similar to those in vivo, and cholinesterase is successfully inhibited in these experiments. In the in vitro gut experiments, on the other hand, the in vivo conditions were not duplicated.

It is seen from the results that the combined (gut plus fat body) converting capacity does not differ consistently between susceptible and nonsusceptible insects (roach 93, mealworm 184, mourning cloak 133, squash bug 74, milkweed bug 111) and therefore no explanation of differential toxicity is given by suggesting equal significance of gut and fat body.

Finally, nerve cords from both classes of insects were effective in converting schradan.

Suggested Hypothesis The following hypothesis is therefore suggested: The only substantial contribution to poisoning is made by the schradan which is converted in the nerve cord. In nonsusceptible insects so much conversion occurs in the fat body that little schradan reaches the nerve cord unchanged. The relative inactivity of the converted schradan produced by the fat body could be due to the failure of the converted material to penetrate a membrane of the type which Hoyle (*74*) has recently shown to invest the nervous system of the locust. Since this membrane was looked for to explain the insensitivity of insect nerves to the high potassium content of the haemolymph, and since the high potassium is universal in insects, it is probable that this membrane is also universal. A similar barrier to converted schradan is shown in mam-

Table I. Toxicity of Schradan

Animal	Mode of Application	Investigator	LD_{50}, γ/G.
Nonsusceptible			
Housefly	Topical	Metcalf and March (*21*)	>500
Bee	Topical	Metcalf and March (*21*)	>100
Colorado beetle	Injection	Duspiva (*7*)	"Massive"
Roach	Topical	Metcalf and March (*21*)	>100
Roach	Injection	O'Brien and Spencer	>100
Mealworm (larva)	Injection	O'Brien and Spencer	>100
Blowfly	Injection	O'Brien and Spencer	175
Mourning cloak (larva)	Injection	O'Brien and Spencer	>400
Susceptible			
Squash bug	Injection	O'Brien and Spencer	16
Milkweed bug	Injection	O'Brien and Spencer	ca. 30
Willow aphid	Topical	Duspiva (*7*)	22
Flame bug	Topical	Duspiva (*7*)	34
Rat	Intraperitoneal injection	Dubois *et al.* (*5*)	8
Guinea pig	Intraperitoneal injection	Dubois *et al.* (*5*)	10

mals, where brain cholinesterase appears to be unaffected by large doses of schradan which inhibit peripheral cholinesterase strongly (*5*). [This property is not observed in any other phosphatic anticholinesterases except those of the schradan type (*6*) and seems to be conferred by the dimethylamide group. As the hepatectomized rat is not susceptible to schradan poisoning, it is to be presumed that schradan either fails to penetrate the mammalian nerve, or it is not capable of being converted there, or both.] Alternatively, the inactivity might be due to the instability of the converted material. If the latter has a stability similar to monochloroschradan, its half-life would be of the order of 40 minutes [schradan has a half-life of about 10 years (*26*)] (*30*).

Because schradan poisoning takes long to develop, sometimes 48 hours, the stability of the intermediate is of some importance. This long course of poisoning substantiates the hypothesis, as the nonnervous tissue is so very active in all insects that if its products contributed to the poisoning, a rapid effect would be expected. Further support is given by the results of experiments which showed that schradan plus mid-gut incubated 24 hours along with cholinesterase gives a much greater inhibition than schradan plus mid-gut incubated together 24 hours followed by cholinesterase addition and assay, 90 minutes later. This suggests that the anticholinesterase is unstable, so that its full effect is obtained only when it can inhibit cholinesterase as fast as it is produced without spatial or temporal hindrance.

When monochloroschradan was applied in corn oil solution to the metathoracic leg of the roach, and its effect upon axonic transmission in the crural nerve observed by use of an oscillograph, no potentiation or block was observed, the only effect being a slight drop in the amplitude of the action potential. Roeder *et al.* (*27*) and Lalonde (*16*) have shown that all anticholinesterases so far tested, except eserine, produce striking effects under these conditions. As monochloroschradan is a powerful anticholinesterase, this suggests that it fails to penetrate the nerve. Of course, monochloroschradan may have very different physical properties from the biologically altered schradan.

The hypothesis is based almost entirely upon observations of the relative converting capacity of various tissues, and the phenomenon of the marked differential toxicity might well be more elegantly explained by some absolute qualitative difference, biochemical or histological.

Table II. Relative Effectiveness per Unit Body Weight of Various Tissues in Converting Schradan

	Nonsusceptible			*Susceptible*	
Mean Weight, Mg.	*Roach* 737 ± 17	*Mealworm larva* 231 ± 7	*Mourning cloak larva*	*Squash bug* 93 ± 6	*Milkweed bug* 50 ± 3
Fore-gut	1.4 ± 0.6				
Mid-gut	18 ± 2	13 ± 3	12 ± 2	63 ± 7	91 ± 10
Hind-gut	19 ± 4				
Fat body	55 ± 6	171 ± 18	81 ± 9	11 ± 2	20 ± 4
Residuum			40 ± 2		
Nerve cord	0.4 ± 0.1	....	....	0.2 ± 0.1	....
Cuticle	0.8 ± 0.1	0	....	0	0
Muscle	0	....	....	0	....

System Responsible For Conversion

The question now arose as to the nature of the system responsible for the schradan conversion. In the following experiments roach mid-guts or hind-guts were used. Because numerous insect tissues contain symbiotic microorganisms, it was desired to find whether the process was microbiological. Washing the tissue in $10^{-3}M$ formaldehyde followed by incubation in $10^{-4}M$ formaldehyde did not affect the system, nor did previous saturation with toluene of the saline solution used in the incubation. But freezing rapidly in solid carbon dioxide destroyed most of the activity, and homogenizing reduced it severely. Tissues allowed to stand at room temperature for 16 hours, with or without added penicillin or streptomycin, lost all activity. All these results indicate that the process is due to a tissue enzyme system, not to a microorganism.

Breis could be activated to a small but significant extent by adding adenosine triphosphate ($5 \times 10^{-4}M$), cytochrome C ($10^{-5}M$), and magnesium chloride ($2 \times 10^{-3}M$). It would be most useful if an active brei could be prepared, and the authors hope to do further work along these lines. Fleisher and Jandorf (*9*) found that liver breis could not activate schradan, although slices were very effective.

Inhibitors

The effects of various inhibitors and treatments were investigated, with the results shown in Table III. The per cent inhibitions are derived from relative effectiveness figures obtained by the procedure given above. The inhibitor pattern is rather odd, in that although the effects of mercuric chloride and iodoacetate suggest the presence of SH groups, chloropicrin, which Mackworth (*17*) has shown to inhibit SH enzymes specifically, is not very effective. Furthermore, cysteine inhibits the system where it might be expected to protect it; it may act as an alternative substrate for some part of the system, and thus be a competitive inhibitor. The effectiveness of azide suggests the presence of a metalloenzyme in the system, possibly cytochrome oxidase, while the malonate inhibition implicates succinic dehydrogenase. However, several of these effects could be explained on the basis of the inhibition of portions of the glycolytic and tricarboxylic cycle pathways, whereby the energy available for the schradan activation is limited. The malonate effect suggests that the process is linked with the tricarboxylic acid cycle and therefore the marked inhibition produced by anaerobiosis cannot be taken to confirm absolutely that the process is a direct oxidation, for the oxygen deficiency could act simply by reducing the available energy.

Table III. Effect of Inhibitors and Treatments on Schradan Activation by Roach Gut

Treatment	*% Inhibition of Schradan Activation*
Iodoacetic acid, $10^{-3}M$	81
Sodium fluoride, $10^{-3}M$	82
Hydroxylamine, $10^{-3}M$	90
Mercuric chloride, $10^{-3}M$	99
Chloropicrin, $10^{-3}M$	11
Sodium azide, $10^{-3}M$	88
Malonic acid, $10^{-3}M$	87
Cysteine, $10^{-3}M$	95
Homogenizing	99
Heat	100
Freezing	98
Anaerobiosis	97

The observed effect of the failure of a compound to inhibit the schradan-conversion system is that the activity of the cholinesterase is greatly lowered by converted schradan produced by the tissue. Clearly, this effect would be produced if the compound inhibited cholinesterase directly. The only case where this situation arose was with chloropicrin. In a separate experiment this compound was shown to be an extremely weak cholinesterase inhibitor, giving only 5% inhibition at $10^{-3}M$. The finding that chloropicrin is a weak inhibitor of the conversion system is therefore not an artifact. Conversely, the results show that mercuric chloride is not an effective inhibitor of serum chlolinesterase, a result which conflicts with that of Goldstein and Doherty (*11*), who were, however, using a purified enzyme preparation.

Metcalf and March (*20*) recently reported a system present in roach gut

which was capable of converting many organic thionophosphates to the corresponding phosphates. This system also was sensitive to homogenization, but its inhibitor pattern differed in that fluoride was ineffective and chloropicrin was highly effective. The system was absent from cuticle, otherwise its distribution in the insect body was very similar to that shown for the schradan-converting system.

Sacktor and Bodenstein (*28*) studied the cytochrome C distribution in the roach and found it to be closely correlated with the degree of tracheation. If schradan were oxidized by some general oxidase, one might expect to find that those tissues which were well tracheated and cytochrome C-rich would make the best conversion. This is not the case—muscle is very high in cytochrome C and excellently tracheated but has no effect on schradan. It seems, then, that we must look for some specific enzyme. The authors' reluctance to suggest *ad hoc* a new system to deal with such an exotic compound as an amidophosphate led to the following admittedly speculative reasoning:

Oxidation Of Schradan

If Hartley's scheme is correct, the activation of schradan is produced by the coordination of an oxygen onto the tertiary nitrogen of the dimethylamido group. The only similar oxidation found in normal metabolism is the oxidation of trimethylamine to its oxide. Now although it appears that trimethylamine oxide is of major importance as a part of the nitrogenous metabolism of fish only (and of these, mainly marine species, *29*), yet Chiancone (*4*) has shown that trimethylamine and trimethylamine oxide are normal urinary constituents in the four mammals he studied. Huerga and Popper (*15*) have shown that if choline is fed to mammals, its nitrogen is excreted as trimethylamine oxide, and that while the breakdown of choline to trimethylamine is made primarily by intestinal microflora, the subsequent oxidation is made in the tissues. Evidence for tissue oxidation of injected trimethylamine was given by Norris and Benoit (*24*). The enzyme which may be responsible for this oxidation, may be called "trimethylamine oxidase" to distinguish it from triamine oxidase, an enzyme which is responsible for the opposite process—i.e., the reduction of trimethylamine oxide to trimethylamine (*23*). The two enzymes may, of course, be identical. So far there has been no demonstration of trimethylamine oxidase in vitro, although triamine oxidase has been demonstrated in bacteria (*23*), yeast (*32*), and various mammalian tissues (*22*).

The possibility exists that trimethylamine oxidase is responsible for the oxidation of schradan. Whole roach gut was taken as a characteristic schradan-converting tissue and incubated for 17 hours with trimethylamine in buffer. Trimethylamine was assayed by Dyer's colorimetric picrate method (*8*). The initial concentration of trimethylamine nitrogen was 0.0116 mg.; after incubation, 0.0026 mg. was found. It remains to be shown that the amine was indeed converted to the oxide (which does not give a color with picrate). Preliminary studies indicated that azide and cysteine ($10^{-3}M$) did not inhibit this conversion, but this may be due to the relatively enormous quantity of tissue which was used (two whole guts with 4 ml. of solution) because of the insensitivity of the chemical determination compared with the highly sensitive enzymic method.

Considering the mammalian system once more, one would expect, from the work which proved that schradan-converting capacity was restricted to the liver, that mammalian trimethylamine oxidase would be similarly restricted. Müller and Immendörfer (*22*) failed to find trimethylamine oxidase in perfused or homogenized liver, although triamine oxidase was found in various tissues, ininclud ing liver. However, if the schradan-converting system is identical with trimethylamine oxidase, this failure is perhaps due to the sensitivity of the system to experimental conditions: Examination for liver trimethylamine oxidase has not been carried out under the conditions found successful for schradan conversion. Schradan oxidation was detected by a highly sensitive enzymic method; this coupled with the fact that the oxidation probably requires energy (since bacteria make use of the conversion of trimethylamine oxide to trimethylamine, presumably as an energy source), may explain the failure to demonstrate trimethylamine oxidase in vitro in mammalian tissues.

Acknowledgments

The authors would like to thank the members of the Entomological Department of this laboratory, especially B. N. Smallman and L. S. Wolfe, for advice on matters entomological. Vincent Lalonde of the Science Service Laboratory, Chatham, Ontario, very kindly carried out the electrophysiological study.

One of the authors (R. D. O'Brien) was in receipt of a Canadian Industries Limited Fellowship.

Literature Cited

(1) Aldridge, W. N., Berry, W. K., and Davies, D. R., *Nature*, **164,** 925 (1949).
(2) Casida, J. E., Allen, T. C., and Stahmann, M. A., *J. Am. Chem. Soc.*, **74,** 5548 (1952).
(3) Cheng, K. K., *Brit. J. Exptl. Pathol.*, **31,** 44 (1951).
(4) Chiancone, F. M., *Boll. soc. ital. biol. sper.*, **15,** 579 (1940).
(5) Dubois, K. P., Doull, J., and Coon, J. M., *J. Pharmacol. Exptl. Therap.*, **99,** 376 (1950).
(6) Dubois, K. P., Doull, J., Okinaka, A. J., and Coon, J. M., *Ibid.* **107,** 464 (1953).
(7) Duspiva, F., *Pflantzenschutz. Tag.*, **70,** 91 (1951).
(8) Dyer, W. J., *J. Fisheries Research Board Can.*, **6,** 351 (1945).
(9) Fleisher, J. H., and Jandorf, B. J., *Federation Proc.*, **11,** 212 (1952).
(10) Gardiner, J. E., and Kilby, B. A., *Biochem. J.*, **51,** 78 (1952).
(11) Goldstein, A., and Doherty, M. E., *Arch. Biochem.*, *33*, 35 (1951).
(12) Hall, S. A., Stohlman, J. W., and Schechter, M. S., *Anal. Chem.*, **23,** 1866 (1951).
(13) Hartley, G. S., XIIth International Congress of Pure & Applied Chemistry, New York, September 1951.
(14) Hoyle, G., *J. Exptl. Biol.*, **30,** 121 (1953).
(15) Huerga, J. de la, and Popper, H., *J. Clin. Invest.*, **30,** 463 (1953).
(16) Lalonde, V., thesis, University of Western Ontario, 1953.
(17) Mackworth, J. E., *Biochem. J.*, **42,** 82 (1948).
(18) Martin, H., *J. Sci. Food Agr.*, **1,** 163 (1950).
(19) Martin, H., *Trans. IXth Intern. Congr. Entomol.*, Amsterdam (1951).
(20) Metcalf, R. L., and March, R. B., *Ann. Entomol. Soc. Amer.*, **46,** 63 (1953).
(21) Metcalf, R. L., and March, R. B., *J. Econ. Entomol.*, **42,** 721 (1949).
(22) Müller, H., and Immendörfer, I., *Z. physiol. Chem.*, **275,** 267 (1942).
(23) Neilands, J. B., *J. Fisheries Research Board Can.*, **6,** 368 (1945).
(24) Norris, E. E., and Benoit, J. B., *J. Biol. Chem.*, **158,** 443 (1945).
(25) Ripper, W. E., Greenslade, R. M., and Hartley, G. S., *Bull. Entomol. Research*, **40,** 481 (1950).
(26) Ripper, W. E., Greenslade, R. M., and Hartley, G. S., *J. Econ. Entomol.*, **44,** 448 (1951).
(27) Roeder, D. D., *Johns Hopkins Hosp. Bull.*, **83,** 587 (1948).
(28) Sacktor, B., and Bodenstein, D., *J. Cellular Comp. Physiol.*, **40,** 157 (1952).
(29) Shewan, J. M., *Biochem. Soc. Symp.*, **6,** 28 (1951).
(30) Spencer, E. Y., and O'Brien, R. D., J. AGR. FOOD CHEM., **1,** 716 (1953).
(31) Stahmann, M. A., Casida, J. E., and Allen, T. C., *Federation Proc.*, **12,** 273 (1953).
(32) Tomita, M., and Tanaka, T., *J. Biochem. (Japan)*, **36,** 345 (1944)

Received for review August 13, 1953. Accepted September 19, 1953. Presented in part at the 36th Annual Conference of the Chemical Institute of Canada, June 4, 1953, Windsor, Ontario. Contribution No. 22, Science Service Laboratory, Department of Agriculture, London, Canada.