Technical Papers

The Lipotropic Properties of Inositol

C. H. Best, C. C. Lucas, J. M. Patterson, and J. H. Ridout

Banting and Best Department of Medical Research
University of Toronto

In the original report (*2*) of the discovery of the lipotropic effect of inositol and in subsequent publications (*3, 4, 7*) it was claimed that inositol exerts a specific effect upon the so-called "biotin fatty liver," which was believed to be the same as that produced by a beef-liver fraction. This was particularly interesting, since the "biotin fatty liver," which was stated to be resistant to choline, was supposed to be characterized by a high content of cholesterol. Inositol was reported to be more effective than choline in reducing the level of cholesteryl esters in the lipides of "biotin fatty livers" and also in those resulting from the feeding of cholesterol (*7*).

Beveridge and Lucas (*1*), in this laboratory, found that under certain dietary conditions, however, inositol was not more, but distinctly less, active than choline in reducing cholesteryl esters in liver lipides. Further work (to be published shortly) has shown that under all the experimental conditions chosen (21 comparative experiments) choline was at least equally, and usually more, effective than inositol in reducing bound cholesterol. It was invariably more active in reducing total lipides. Similar results were obtained in rats fed on fat-free diets, on diets containing fat, and on diets containing fat and *cholesterol*. When biotin was injected, choline was distinctly more active in reducing total lipides and was at least as effective as inositol in reducing bound sterols. In the prolonged experiments choline was much more effective than inositol in lowering the cholesteryl esters whether or not biotin was given.

No evidence has been obtained in this laboratory to suggest that there is any difference between the fatty liver produced by biotin and that caused by a high fat diet. The ratio of bound sterol to glycerid is the same in the presence of biotin as in its absence. Groups of rats (usually about 12 animals on each diet) injected with biotin (5 γ daily) responded just as well to choline as did those not receiving biotin. The biotin and nonbiotin groups responded to about the same extent to administration of inositol, this response being less than that produced by an equal quantity of dietary choline. The combined effect of choline and inositol is equally pronounced whether biotin is present or absent. In a recent paper, McHenry (*5*) has retracted certain of his earlier statements concerning (a) the characteristics of the "biotin fatty liver" (*i.e.* its identity with that produced by feeding a certain liver fraction) and (b) the specific lipotropic properties of inositol on this "biotin fatty liver." Our experimental results, which confirm and extend McHenry's latest findings, lead us to advance further along this pathway and to conclude that there is no evidence that the "biotin fatty liver" exists as a unique phenomenon.

McHenry (*5*) has stated that "it is obvious that inositol is effective in preventing the fatty liver caused by the beef liver fraction, while choline is not." That the liver fraction contains choline[1] has been noted (*6*), but the fact seems to have been overlooked in explaining the apparently greater relative lipotropic effect of inositol than choline upon the fatty livers so produced. Addition of further choline had only a small effect, as might be expected. Addition of inositol produced the well-known synergistic lipotropic effect. McHenry (*5*) has stated that "the effect of a combination of choline and inositol is similar to that produced by the same amount of inositol alone." However, the inositol was *not alone* in his experiments, because the liver fraction which was fed contained large amounts of choline. It is obvious that the fatty liver produced by the liver fraction is somewhat resistant to choline, but McHenry's failure to mention the presence of choline in the liver extract has tended to overemphasize the role of inositol under these particular circumstances. It would be interesting to know the level of liver fat which would result from giving a choline-free liver fraction and to compare the relative effects of choline and inositol under such conditions. Experiments along these lines are at present being conducted.

We have conducted many experiments in which diets containing cholesterol were fed over periods from 3 to 16 weeks and have not noted any preferential effect of inositol in lowering either bound sterols or total lipides. In fact, the lipotropic effect of inositol, which was less than that of choline even in short-term "cholesterol" experiments, diminished in relation to that of choline as the experiments were prolonged. In contrast, little, if any, diminution in the choline effect was observed during 16 weeks.

[1] We are using a similar fraction, obtained from the same source, and by the ennea-iodide procedure have found 9 mg. per cc. of free choline and 24 mg. total choline. The latter figure has been confirmed by microbiological assay.

It may also be recorded here that inositol (30 mg. per day) has not, in our experience, been effective in preventing the occurrence of the hemorrhagic kidneys which develop in young rats on diets low in choline and methionine.

References

1. Beveridge, J. M. R., and Lucas, C. C. *J. biol. Chem.*, 1945, **157**, 311.
2. Gavin, E. G., and McHenry, E. W. *J. biol. Chem.*, 1941, **139**, 485.
3. Gavin, E. G., and McHenry, E. W. *J. biol. Chem.*, 1941, **141**, 619.
4. Gavin, E. G., Patterson, J. M., and McHenry, E. W. *J. biol. Chem.*, 1943, **148**, 275.
5. MacFarland, M. L., and McHenry, E. W. *J. biol. Chem.*, 1945, **159**, 605.
6. McHenry, E. W., and Gavin, E. G. *J. biol. Chem.*, 1940, **134**, 683.
7. McHenry, E. W., and Patterson, J. M. *Physiol. Rev.*, 1944, **24**, 128.

TDE, 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethane, as an Anopheline Larvicide

Christian C. Deonier and Howard A. Jones

Bureau of Entomology and Plant Quarantine[1, 2]

U. S. Department of Agriculture

Compounds related to DDT, including those present in the technical product, have been tested for their toxicity to fourth instars of *Anopheles quadrimaculatus* Say. One of these compounds, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane,[3] hereinafter called TDE from the generic name "tetrachlorodiphenylethane," has been found to have a toxicity equal to, and in some forms of application greater than, that of DDT. Tests have been made to compare the toxicity of DDT and TDE when applied in acetone suspensions, dusts, and oil solutions. The methods used in testing acetone suspensions and dusts were those described by Deonier, *et al.* (*J. econ. Ent.*, 1945, **38**, 241–243).

Acetone suspensions. Tests comparing the initial kill of acetone suspensions of TDE and DDT are reported in Table 1. In this form the two compounds were not significantly different in effectiveness. TDE, however, is indicated to be better than DDT in its residual toxicity, as shown in Table 2.

In a comparison at 0.01 p.p.m., the average length of time required for complete knock-down of larvae was 0.81 hour for DDT and 1.166 hours for TDE.

Dusts. Table 3 shows that, when impregnated on talc and applied as a dust, TDE had a toxicity to *Anopheles quadrimaculatus* equal to, or greater than, that of DDT. In other tests TDE has shown some indication of being superior against culicine larvae.

TABLE 1

Comparative Toxicity of TDE and DDT in Acetone Suspensions to Fourth Instars of *Anopheles quadrimaculatus*
(20 larvae per test, 3 replications)

Material		Mean mortality in:	
		24 hours	48 hours
	p.p.m.	%	%
TDE	0.0050	88.3	100.0
	.0033	68.3	93.3
	.0025	58.3	95.0
DDT	0.0050	83.3	96.6
	.0033	73.3	93.3
	.0025	55.0	81.6

TABLE 2

Comparative Stability of TDE and DDT When Applied in Acetone Suspensions at 0.01 p.p.m. Against Fourth Instars of *Anopheles quadrimaculatus*
(20 larvae per test, 3 replications)

Material	Time between treatment and introduction of larvae	Mean mortality in:	
		24 hours	48 hours
	Days	%	%
TDE	0	98.6	100.0
	2	93.3	100.0
	4	100.0	....
	7	93.3	98.3
	9	43.3	61.6
DDT	0	100.0	....
	2	55.0	75.0
	4	3.0	8.3

Fuel-oil solutions. One of the difficulties encountered in laboratory comparisons of DDT in oil solutions is that the toxicity of the oil may affect the results where it is used in appreciable quantities. The smallest amount of oil that can be accurately measured is toxic when applied to a beaker or a pan. A spray chamber has been constructed in which larvae in small containers are exposed to small amounts of atomized spray. By dispersing a small quantity of spray in the spray chamber, larvae can be exposed to sublethal dosages of DDT or other materials in solutions.

In a spray chamber (8 × 8 × 8 ft.), 0.4 ml. of No. 2 fuel oil containing 0.5 per cent of DDT gave an average mortality of 76.6 per cent in 48 hours in three containers exposed simultaneously. Paired tests of TDE used at the same dosage gave 100 per cent mortality in 24 hours.

In further tests against fourth instars of *Anopheles quadrimaculatus,* the mean mortality of 0.4 ml. of No. 2 fuel oil containing 0.5 per cent of DDT was 55.8 per cent after 24 hours and 69.1 per cent after 48 hours. Against the same larval population 0.2 ml. of No. 2 fuel oil containing 0.5 per cent of TDE gave

[1] This work was conducted under a transfer of funds, recommended by the Committee on Medical Research, from the Office of Scientific Research and Development to the Bureau of Entomology and Plant Quarantine.

[2] Acknowledgment is made to Sgt. Harry H. Incho for his valuable assistance in conducting the laboratory tests.

[3] The term "DDD" has been used for this compound, but the designation "TDE" seems preferable. The latter is used in order to avoid possible phonetic confusion with DDT and also because two proprietary products called DDD are on the market.