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# COMMENTARY

## IMMUNOPHARMACOLOGICAL CONSIDERATIONS IN REYE'S SYNDROME: A POSSIBLE XENOBIOTIC INITIATED DISORDER?

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Reye's syndrome may be described as an encephalopathy associated with fatty degeneration of the viscera which occurs primarily in children [1–6] although a few cases have been reported in young adults [6]. Several similar hepatic encephalopathies have long been recognized [7, 8]. As a clinically observed childhood disorder however, Reye's syndrome is a relatively new phenomenon which has become increasingly widespread over the past fourteen years [1–6]. The aetiology of this syndrome remains unknown although a link with viral, particularly influenza B, infections may exist [1–3, 6]. Other workers maintain that a specific urea cycle enzyme deficiency occurs in susceptible individuals [9]. Of greater relevance to the present article is the suggestion that various exogenous compounds may be involved in the pathogenesis of this disorder, including; aflatoxins [10–12], salicylates [4], pentenoic acids [13, 14], and pesticide chemicals [15, 16]. The complicated metabolic abnormalities seen clinically in Reye's syndrome have been recently reviewed [17].

The purpose of the present article is to evaluate some recent laboratory, clinical and epidemiological findings relevant to Reye's syndrome from a biochemical and immunopharmacological perspective. Special emphasis is placed on the possibility that environmental exposure to different, potentially toxic, foreign compounds (xenobiotics) may be responsible for the early pathogenesis of this disorder. In this context therefore, various chemically diverse compounds (or their toxic biotransformation products) will be considered to be *xenobiotic initiating factors*, capable of altering the body's biochemical and immunological status, thereby rendering it more susceptible to (viral) infection.

*Animal models.* In recent years various animal models have been used to investigate possible aetiological factors in Reye's syndrome. Prompted by the occurrence of several cases of the syndrome in the Atlantic provinces of Canada, Crocker *et al.* developed an experimental model which demonstrated that certain insecticides [15], or other constituents of insecticide spray mixtures [16], can increase the lethality of subsequent viral infection. Different compounds have been studied similarly using other mammalian species [10, 13, 14]. Earlier research had shown an interaction between viral infections and polychlorinated biphenyls in the duck [18]. To test the hypothesis developed in the present paper such models require further utilization with a much wider range of suspected xenobiotics including concomitant immunological assessments. Some of the interesting results published to date will be reconsidered from the immunopharmacological viewpoint promulgated here.

In their original paper [15], Crocker *et al.* demonstrated that the mortality rate due to subcutaneously injected mouse encephalomyocarditis virus was markedly greater in newborn animals previously painted (daily for nine days) with a cornoil solution of dicophane (DDT) plus fenitrothion compared to groups treated with either chemical alone. The animals that succumbed developed convulsions before death and histological examination revealed fatty infiltration of the liver. Groups pretreated with DDT prior to the viral administration showed large fatty droplets in the central lobular region of the liver, while finer and more generally distributed hepatic fat deposits were observed in the fenitrothion and fenitrothion plus DDT treated animals [15].

In a more recent paper [16], it was shown that it is the emulsifying agents used commercially in insecticide spray solutions which may be responsible for the increased lethality following later viral infection. In fact, this work indicated that the presence of fenitrothion in the pretreatment solutions actually protected the animals against the enhanced viral susceptibility induced by the solvent-emulsifier solutions alone. This latter finding is particularly interesting in the context of the present paper.

The authors offered no explanation for the apparent protective effect of fenitrothion in these studies but did suggest possible virus–chemical interactions to account for the enhancing effect of chemical pretreatment on susceptibility to viruses generally [16]. Two plausible immunopharmacological points were not made. Firstly, there was no mention of possible competitive interactions of the chemicals involved for biotransformation enzymes to account for the protective effects of fenitrothion. Secondly, the increased mortality due to viral infection in the pretreated animals was not considered in terms of the potential adverse effects of xenobiotics in general on the integrity and function of the immune system.

*Biotransformation aspects.* In general all highly lipophilic xenobiotics (all compounds foreign to the body including drugs) are rendered more water soluble and hence, more readily excreted, by the so-called mixed-function oxidase (microsomal) enzyme system located in the hepatic smooth endoplasmic reticulum [19]. In this way many lipid soluble foreign chemicals are detoxified. However, it is becoming increasingly evident that some compounds may be metabolized to pharmacologically active [20–22], or even highly

toxic [23–28], intermediates by components of this same enzyme system. In mammals it is well known that many environmental xenobiotics, including DDT [19, 29, 30], 3,4-benzpyrene [19, 23, 31] and polychlorinated biphenyls [32] are powerful inducers of the mixed-function oxidase system resulting in an enhanced biotransformation capacity. Enzyme induction is also associated with an increase in liver weight and microscopically visible smooth endoplasmic reticulum [19, 33]. (Proliferation of the smooth endoplasmic reticulum has been reported in cases of Reye's syndrome [2].) Conversely, some compounds, including the organophosphates, although metabolized by components of this enzyme system, may competitively inhibit the concomitant biotransformation of other xenobiotics [19, 34, 35]. Certain xenobiotics may be highly toxic to very young animals (depending on species) due to underdevelopment of the mixed-function oxidase enzymes responsible for detoxification [36]. Biotransformation mechanisms may prove useful in interpreting the animal experiments of Crocker *et al.* [15, 16], especially with regard to the protective effects afforded by fenitrothion mentioned above.

As with other organophosphate insecticides, the anticholinesterase activity of fenitrothion is actually due to its *in vivo* biochemical conversion to an active oxygenated derivative, fenitrooxon [21, 22, 35]. However, as outlined in Fig. 1, recent studies [35] have shown that fenitrothion, but *not* fenitrooxon inhibits the metabolism of other xenobiotics both *in vitro* and *in vivo*. Different types of inhibition were observed *in vitro* (fenitrothion added to the microsomal preparation) compared to *in vivo* (fenitrothion pretreatment of the animals before sacrifice) experiments, being competitive and noncompetitive, respectively. On the basis of the prolongation of hexobarbitone induced sleeping times, prior fenitrothion treatment was as effective as the "classical" mixed-function oxidase inhibitor SKF 525-A (*β*-diethylaminoethyldiphenyl-*n*-propylacetate HCl). Interestingly, *in vivo* experiments showed a species difference– inhibition in mice was markedly greater than in rats [35].

On the basis of the foregoing an explanation of the protective effect observed with fenitrothion in the chemically pretreated, viral infected animals is possible. Fenitrothion, by inhibiting the mixed-function oxidase enzymes, prevented the accumulation of toxic metabolites otherwise produced by the action of these enzymes on certain absorbed constituents of the solvent-emulsifier preparation. (Although the exact composition of the solvent-emulsifier preparation is not precisely known, analysis indicated the presence of several aromatic compounds [16].) Such an interpretation is consistent with the fact that known inhibitors of the mixed-function oxidase system protect against the toxicity of carbon tetrachloride and other organic hepatotoxins [27, 37]. DDT and similar microsomal enzyme inducers however, would be expected to provide no such protection and might even promote the toxicity of the offending agent(s). The validity of such interpretations could be readily tested in the animal model of Crocker *et al.* [16] by using other known inhibitors and stimulants of the microsomal enzyme system, such as SKF 525-A (or piperonyl butoxide) and phenobarbitone respectively [27, 33, 35], in conjunction with the suspected solvent-emulsifier preparations. It is assumed in this animal model, and in Reye's syndrome itself, that underlying biochemical anomalies are initiated by exposure to a xenobiotic factor(s), which in turn results in an impairment of normal immune defences against subsequent (viral) infections.

*Biochemical and immunological aspects.* The essential biochemical abnormalities associated with Reye's syndrome include: (i) altered lipid metabolism with diffuse hepatic triglyceride deposits and increased serum free fatty acid concentrations, (ii) hypoglycaemia, and (iii) impaired amino acid and urea cycle metabolism as manifested by high ammonia and low blood citrulline concentrations [2, 17]. The pronounced CNS symptoms, such as convulsions and coma may be due to any one, if not all, of these biochemical changes.

The observed fatty degeneration and structural changes of the liver are congruous with the hypothetical initial involvement of xenobiotic factors in the pathogenesis of this syndrome. Several known hepatotoxic xenobiotics, including ethanol, also produce obvious changes in lipid metabolism and liver structure in laboratory animals [10, 37, 38].

Particularly relevant to an immunopharmacological basis of Reye's syndrome as proposed here are the numerous recent reports demonstrating that many drugs and other xenobiotics can modify the *in vitro* lymphocyte response to mitogens [39–41] and other stimuli [42] as well as the enumeration of T and B cells [43, 44]. On the other hand, it is conceivable that impaired immune responsiveness could be indirect or secondary to xenobiotic induced changes in fatty acid metabolism and mobilization [2, 11, 17]. Recent investigations in several laboratories have demonstrated diminished lymphocyte function in the presence of certain (unsaturated) fatty acids [45–47]. Moreover, since high plasma cyclic AMP (this "second messenger" promotes intracellular lipolysis [48]) concentrations have been found in patients with Reye's syndrome [49], it is noteworthy that this nucleotide has a myriad of effects (mostly inhibitory)

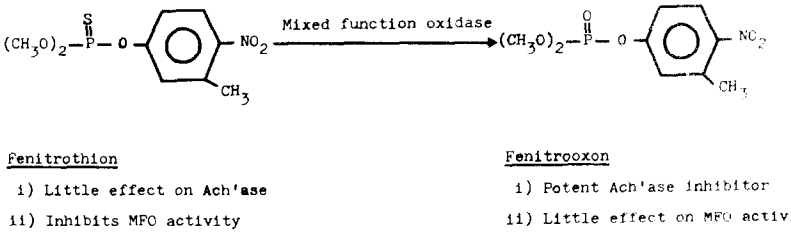


Fig. 1. The relative effects of the organophosphate pesticide fenitrothion and its metabolite, fenitrooxon on mixed-function oxidase (MFO) and acetylcholinesterase (Ach'ase) enzymes (based on [35]).

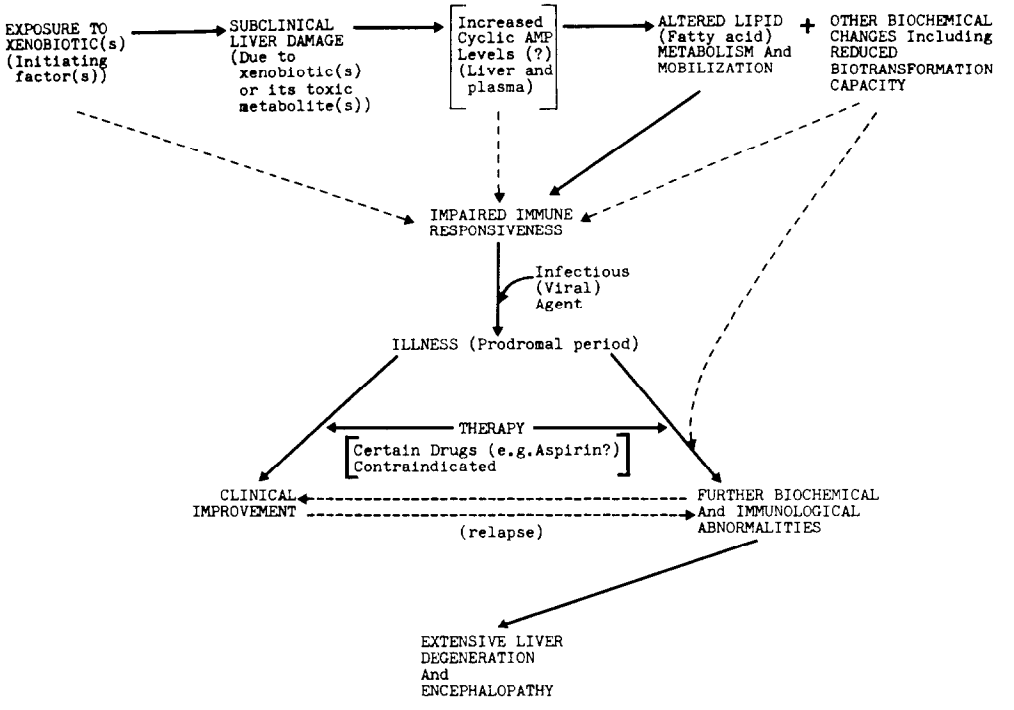


Fig. 2. An outline of the postulated xenobiotic initiated pathogenic mechanisms in Reye's syndrome. (For details, see text).

on the immune system [44, 50]. Also, in addition to parenchymal cell changes, it is possible that a xenobiotic induced malfunction of hepatic reticuloendothelial (Kupffer) cells could partly contribute to an overall modification of immunological function. Overloading of the phagocytic capacity of damaged Kupffer cells by enteric bacteria has been postulated to account for the hyperglobulinaemia observed in cirrhosis, by allowing excess antigen contact with antibody forming (B) cells [51]. Increased numbers of "young" Kupffer cells are thought to occur in Reye's syndrome [2] and in experimental animals after $CCl_4$ induced hepatotoxicity [51].

The postulated xenobiotic initiated pathogenic mechanisms presented here are outlined in Fig. 2.

*Possible xenobiotic factors in Reye's syndrome.* In the present work it is suggested that Reye's syndrome can be precipitated by many chemically unrelated compounds, each of which has the capacity to cause subclinical biochemical and functional abnormalities of the liver and immune system [Fig. 2]. Reye's syndrome or very similar clinical disorders are found in many different geographical regions of the world. Thus, animal [10, 13] and clinical studies [11, 12] have implicated the potent carcinogenic and hepatotoxic aflatoxins in the pathogenesis of this syndrome in countries where certain foodstuffs are found to be contaminated with these mycotoxins. Amanitatoxin induced mycetismus [13, 52] and Jamaican vomiting sickness caused by hypoglycin-A in ingested ackee fruit [1, 2, 13, 14, 53] both closely resemble Reye's syndrome. Although a link with viral infections remains unknown in these disorders, it is interesting that the aflatoxins and hypoglycin-A (α-aminomethylenecyclopropylpropionic acid) seem to require biotransformation in the body to produce their toxic effects [25, 26, 53].

As already mentioned, pesticides and various other organic compounds have been investigated as possible aetiological factors in Reye's syndrome [10–16]. Reports have provided evidence that many such compounds can produce definite changes in both liver biochemistry [10, 13–16] and the immune response [41, 54, 55].

The reported association of Reye's syndrome with salicylate ingestion [4] may merely reflect a propensity to use this common drug during the prodromal period. Nevertheless, such pharmacological agents could conceivably exacerbate the condition as suggested in Fig. 2. Salicylic acid and its derivatives have been shown to alter immune function both *in vitro* and *in vivo*. [39, 56, 57] Also, salicylates are highly bound to plasma proteins and are capable of displacing many other compounds from their protein binding sites [58, 59]. Concomitant administration of salicylates or other acidic drugs, therefore, could lead to elevated circulating unbound (active) concentrations of certain fatty acids (the converse—fatty acid displacing drug—may be more likely), amino acids, bilirubin and, if present, other xenobiotics [58–60]. Compounded with this, in advanced stages of the disease toxic drug levels might readily occur as a result of a diminished capacity of damaged hepatocytes to detoxify foreign compounds [19, 27].

Regarding the increase in cases of Reye's syndrome in North America in recent years, it is tempting to speculate as to possible xenobiotic initiating factors consistent with the pathogenic mechanisms postulated in this paper. Due to strict food processing regulations and proper storage facilities it seems unlikely

that aflatoxins would be significantly involved in this area of the world and therefore other environmental xenobiotic substances should be considered.

Bearing in mind the young age group in which Reye's syndrome occurs it is important to establish whether or not the flame retardant chemicals added to childrens' clothing materials could in any way be associated with this disorder [61]. The main chemical employed as a flame retardant for childrens' pyjama material is Tris (2,3-dibromopropyl) phosphate which recent research has shown to be a potent mutagen and potential carcinogen following hepatic microsomal metabolism [61, 62]. Both animal and human studies indicate that this compound can be absorbed after topical application or contact [61–63]. Other organic phosphate esters have been reported as contaminants of plastic containers [64, 65].

Epidemiological studies [3, 6] have shown that a nationwide outbreak of Reye's syndrome occurred in the United States between January and April, 1974, reaching a peak in mid-February at about the same time as did the number of reported influenza B cases. It may be noteworthy that this particular outbreak of Reye's syndrome also occurred approximately six months after the inadvertent addition of polybrominated biphenyls (PBB) to the food chain in the state of Michigan [61, 66]. (A lag period between xenobiotic exposure and Reye's symptoms subsequent to viral infection is not inconsistent with the present hypothesis). It is now known that vast quantities of PBB contaminated dairy and poultry products were consumed by humans, particularly in rural areas of Michigan and neighbouring states [61, 66]. An association between the number of Reye's syndrome cases in early 1974 and the PBB incident would be difficult to establish and therefore, at present, must be regarded as mere speculation. Nevertheless, it is known that this outbreak (as admittedly, were earlier ones) occurred in rural and suburban areas with a frequency more than double that in urban areas [3, 6]. Preliminary results however, indicate that in Michigan at least, there was no difference in the prevalence of influenza B infections between rural and urban areas [6]. Forty-six cases of Reye's syndrome were reported in Michigan during the January to mid-April, 1974 period compared to approximately one-third of this number over the previous two year period [67].

*Concluding comments.* Due primarily to the multiplicity of viral agents isolated from patients, the higher incidence in nonurban areas, and the predominance of the disorder in children, several workers have implicated a role for additional environmental factors in the aetiology of this syndrome [1, 2, 6, 10–16]. In view of the suspected link between environmental xenobiotics and viral infections, it is surprising, however, that there is a distinct paucity of information pertaining to the immune response in Reye's syndrome.

Although the immunopharmacological concept propounded in this paper may be controverted eventually, it is the author's opinion nonetheless, that a concerted research effort into the rôle of both xenobiotics and the immune response in Reye's syndrome is now warranted. It is, after all, only recently that the scientific community has seriously begun to consider the increasing evidence that many human cancers are induced by specific xenobiotic agents [68].

Only a few of numerous possible xenobiotics consonant with an immunopharmacological basis of Reye's syndrome have been mentioned here. Moreover, since modern man is exposed to a composite of various exogenous chemicals, chiefly iatrogenic or environmental in origin, the overall concept of xenobiotic initiating factors in this syndrome is further complicated by several conceivable interactive mechanisms, either potentiating or inhibitory in nature. The future task of relating human disease to xenobiotic exposure will not be a simple one. Drugs and their metabolites for instance, have already been detected (in quantities of several kg per day) in city sewage effluents discharged in American rivers, raising the prospect of iatrogenic agents as environmental pollutants [69].

An immunopharmacological basis for Reye's syndrome requires substantiation by means of further epidemiological and animal studies. Researchers, however, should be ever aware of possible "interdisciplinary interactions", especially at the biochemical, pharmacological and immunological levels.

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