Biochemical Pharmacology, Vol. 38, No. 3, pp. 385–390, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

COMMENTARY

'A'-ESTERASES

ENZYMES LOOKING FOR A ROLE?

MICHAEL I. MACKNESS*
Department of Physiology and Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ, U.K.

In 1953, Aldridge classified esterases into two groups dependent on their interaction with organophosphorus anticholinesterases [1, 2]. 'A'-esterases hydrolyse such compounds, whereas 'B'-esterases, which include the carboxylesterases and cholinesterases, are inhibited by them. In the case of 'B'-esterases, the dephosphorylation is very slow, and the enzymes are effectively inhibited by the organophosphate 'suicide' substrate. However, with 'A'-esterases the turnover rates are high; therefore, reactivation is very rapid, leading to the regeneration of free enzyme (Fig. 1).

'A'-esterases are currently classified by the Nomenclature Committee of the International Union of Biochemistry (NC-IUB) as arylesterases (EC 3.1.1.2 [3]). However, recent evidence casts doubt on this classification [4]. In a study of the hydrolysis of paraoxon† and pirimiphos-methyloxon ('A'-esterase substrates) and phenyl-acetate (arylesterase substrate) by the sera of fourteen species of bird representing seven orders and eleven species of mammal representing five orders, ten species of bird had no 'A'-esterase activity and the remaining four only low activity. Nevertheless, all the birds showed considerable arylesterase activity. The mammals studied showed both 'A' and arylesterase activities. From this and other data (see later), it appears that a distinction should be made between these two enzyme activities.

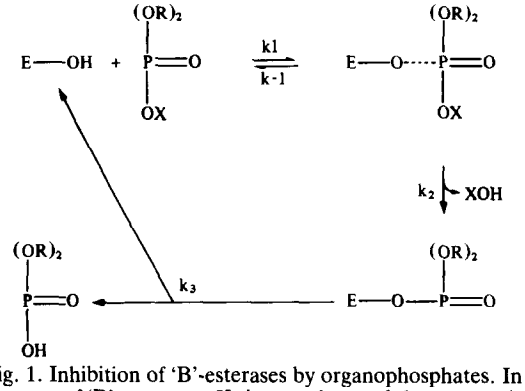

Fig. 1. Inhibition of 'B'-esterases by organophosphates. In the case of 'B'-esterases, K_3 is very slow and the enzyme is effectively inhibited. 'A'-esterases also cause the hydrolysis of organophosphates, but it is not clear whether they do so by the same mechanism. Whatever the mechanism, any complex formed between organophosphate and enzyme is unstable, and the enzyme is rapidly reactivated with such substrates as chlorpyrifos-oxon, diazoxon and pirimiphos-methyloxon. This is indicated by the large turnover number of 18,000 for a purified form of sheep serum 'A'-esterase with chloropyrifos-oxon as substrate (M.I. Mackness, unpublished observations). Key: E-OH, esterase; R usually an alkyl group; and X, leaving group.

'A'-esterases can be subdivided into two groups: (1) Those that are capable of hydrolysing diisopropylfluorophosphate (DFP) and related compounds (including the nerve gases tabun and sarin). These enzymes are dependent on Co^{2+}, Mg^{2+} and Mn^{2+} as metal-ion cofactors and are, in general, termed DFPases (EC 3.8.2.1). (2) Those that hydrolyse paraoxon (*O,O*-diethyl-*O*-*p*-nitrophenyl phosphate) and related organophosphate pesticides and are dependent on Ca^{2+} as a metal-ion cofactor. It is with this latter group and particularly those members found in the blood that this manuscript will be concerned.

'A'-esterase activity is found in a wide variety of organs with liver and blood generally having the highest activities [2, 4, 5, 6]. Mammals have the highest levels of activity in all organs; other animal groups, e.g. birds, fish and insects, have little or no activity. The enzymes catalyse the hydrolysis of an organophosphate ester into its constituent acid and

* Present address: Department of Medicine, University of Manchester, The Royal Infirmary, Manchester M13 9WL, United Kingdom.

† Chemical names of organophosphates referred to in text: chlorothion-oxon = *O,O*-dimethyl-*O*-(3-chloro-4-nitrophenyl) phosphate; chlorpyrifos-oxon = *O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphate; Coroxon = *O,O*-diethyl-*O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl)phosphate; diazoxon = *O,O*-diethyl-*O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphate; dicapthoxon = *O,O*-dimethyl-*O*-(6-chloro-4-nitrophenyl) phosphate; EPN-oxon = *O*-ethyl,phenyl-*O*-(4-nitrophenyl) phosphate; fenitroxon = *O,O*-dimethyl-*O*-(3-methyl-4-nitrophenyl) phosphate; methyl-paraoxon = *O,O*-dimethyl-*O*-(4-nitrophenyl) phosphate; paraoxon = *O,O*-diethyl-*O*-(4-nitrophenyl) phosphate; and pirimiphos-methyloxon = *O,O*-dimethyl-*O*-(2-(diethylamino)-6-methyl-4-pyrimidinyl) phosphate.

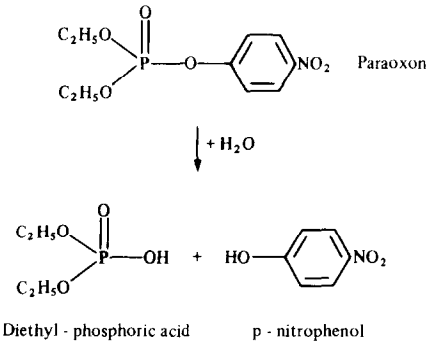

Fig. 2. Hydrolysis of paraoxon.

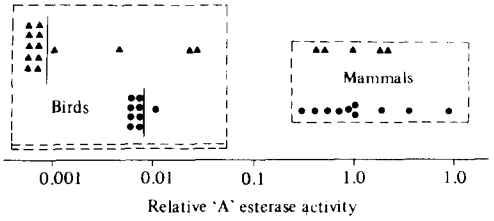

Fig. 4. Serum 'A'-esterase activities of birds and mammals. Activities were originally measured as nmoles product ml serum^{-1} min^{-1} but were converted to relative activities (male rat = 1) and plotted on a log scale. Each point represents a mean value for a single species. Substrates: paraoxon (●), and pirimiphos-methyloxon (▲). Vertical lines indicate limits of detection, and all points plotted to the left of them are for species in which no activity was detected. Activities for the male rat were 61 ± 4 and 2020 ± 130 for paraoxon and pirimiphos-methyloxon respectively. Reproduced with the approval of the British Crop Protection Council.

alcohol (Fig. 2). This reaction requires Ca^{2+} as a cofactor and the pH optimum is alkaline [6, 7].

Role of 'A'-esterases in determining the selective toxicity of organophosphates

As mentioned previously, 'A'-esterases hydrolyse organophosphate anticholinesterases. These compounds are widely used as pesticides (mostly insecticides), and a few of them are nerve gases [8]. They are usually applied as the relatively non-toxic sulphur derivatives (thions) and are 'activated' within the body to the oxidised (oxon) form by the action of microsomal monooxygenases [9]; this process is known as oxidative desulphuration (Fig. 3). It is these oxon forms that are both potent anticholinesterases, causing inhibition of synaptic acetylcholinesterases which eventually leads to death, and substrates for 'A'-esterases. Much of the 'activation' of these thions is thought to occur in the liver, the active oxon then being transported via the blood to the brain, the site of action in vertebrates. Not many cases have been studied in detail. The process will depend, to some extent, on compound, formulation and route of administration. The predominance of liver metabolism tends to be asssumed because of the high metabolic capacity and the early receipt of orally absorbed organophosphates via the hepatic portal system. The presence of high levels of an enzyme in the blood capable of detoxifying these compounds is an important safeguard against poisoning.

Biochemical studies upon sheep serum 'A'-esterases have indicated that several forms of the enzyme exist, associated with the high-density lipoprotein (HDL) fraction of the serum [10, 11]. During ultracentrifugation, sheep serum 'A'-esterase activity and HDL-cholesterol separate into the lipoprotein fraction in the same time-dependent manner [11]. The enzyme has a molecular weight of 360,000 as determined by gel filtration [12] which is similar to that of human HDL_2 [13]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of enzyme fractions shows the presence of several peptides with molecular weights similar to those of the apolipoproteins of HDL [10]. The multiple enzyme forms show differential activity towards such substrates as paraoxon, pirimiphos-methyloxon, coroxon, chlorpyrifos-oxon and diazoxon ([10], and M. I. Mackness, unpublished observations). The picture that emerges is of a family of HDL particles with 'A'-esterase activity, all with different but overlapping substrate specificities towards a range of organophosphate (OP) substrates.

'A'-esterases are of considerable importance in determining the selective toxicity of organophosphates [14]. Rats injected with partially purified rabbit 'A'-esterase were found to be considerably less susceptible to paraoxon poisoning than controls not given 'a boost' in their 'A'-esterase [15].

In two detailed studies on the effects of metabolism on the selective toxicity of pirimiphos-methyl [16] and diazinon [17, 18], neither the rate of production of oxon metabolites nor the degradation of the parent compound in the livers of a range of avian and mammalian species could be correlated with the greater susceptibility of birds to poisoning by these two compounds. In the rat liver, hydrolysis of pirimiphos-methyloxon was much faster than in the liver of the Japanese quail [16]. However, both

Fig. 3. Oxidative desulphuration of parathion. The process of oxidative desulphuration is carried out by microsomal monooxygenases. The reaction results in the formation of the toxic oxon metabolite from the comparatively non-toxic thion and is therefore termed 'activation.'

groups found that the hydrolysis of the active oxon forms was extremely rapid in mammalian serum but either very slow or undetectable in avian sera [16–19]. This is illustrated in Fig. 4. Both groups concluded that the presence of the serum 'A'-esterase activity in mammals was responsible for the different toxicity of these OPs in mammals compared to birds.

The presence or absence of a particular form of the enzyme in a species or individual, therefore, could profoundly affect the toxicity of the organophosphate(s) hydrolysed by that enzyme form to those individuals or groups lacking the form. At this time, however, there is no evidence to support this hypothesis.

Studies performed upon the esterase activity of resistant insects have found no evidence for 'A'-esterase activity. Three strains of the rust red flour beetle (*Tribolium castaneum*), one of them susceptible to OPs, the other two resistant [20], showed considerable 'B'-esterase activity towards three general esterase substrates. None of the strains showed measurable paraoxon or pirimiphos-methyloxon hydrolysis. In a study of strains of the aphid *Myzus persicae* which are resistant to OPs, an esterase was isolated which plays an important role in resistance [21, 22]. However, the esterase proved to be of the 'B'-type. This was also found to be the case with an esterase from the green rice leafhopper (*Nephotettix cincticeps*) which contributed to resistance [23]. Thus, it would appear that insects resistant to OPs cannot call upon 'A'-esterase activity to contribute to their resistance.

Human serum 'A'-esterases

Human serum 'A'-esterase activity, like the activity of sheep serum, is associated with HDL. It shows the same flotation characteristics into the lipoprotein fraction as HDL-cholesterol [11] and can be distinguished from arylesterase activity. Gel filtration of human serum results in the separation of arylesterase activity into three distinct peaks and 'A'-esterase activity into two peaks [4]. These enzyme peaks are distinct from one another, indicating that different enzymes are responsible for these two activities in human serum. In addition to this evidence, serum 'A'-esterase activity is biphasically distributed in caucasian populations (see later); however, in these populations arylesterase activity is unimodally distributed [24, 25]. These findings indicate that the two enzyme activities are different and that the current classification of these enzymes should be altered.

Polymorphism of enzyme activity in human populations. In European and North American caucasian populations, serum 'A'-esterase activity towards paraoxon (paraoxonase) is bimodally distributed [26–31]. One group of workers claim a trimodal distribution in German populations [32, 33]; however, doubt has been expressed as to the validity of the methodology used in these particular studies [31]. Genetic studies suggest that these distributions are determined by two common alleles at a single autosomal locus [27, 30], and interest has developed in the use of this enzyme as a genetic marker in human populations.

The polymorphic distribution of paraoxonase shows great inter-ethnic variability [34, 35]. Thus, individuals with low paraoxonase activity account for some 53% of the European population. This percentage becomes much less in populations far removed from Europe, which are of different ethnic composition from that of Europeans. Thus, Aborigines, Maories and South American Indians have no homozygote low paraoxonase individuals and several populations show a normal distribution of activity [27, 34, 36]. The distribution of paraoxonase in caucasian populations follows the Hardy–Weinberg rule for a two-allele model; however, this is not the case for negroid or mongoloid populations [37] where the Hardy–Weinberg rule for two-allele or three-allele models does not apply. There is some evidence for the presence of multiple forms of serum 'A'-esterase activity in both sheep [7] and humans [4]. The effect of multiple forms of the enzyme activity on the distribution in human populations is at present unknown (as are possible environmental factors which could cause activation, induction or inhibition of the enzyme activity). It is possible, however, that the presence of multiple forms may mean that the genetics of serum paraoxonase distribution may not be as simple to model as previously believed.

The polymorphisms are also substrate dependent, further indicating the possible effects of multiple enzyme forms; paraoxon, methyl-paraoxon, chlorothion-oxon and EPN-oxon all give rise to polymorphic distributions, whereas dicapthoxon, fenitroxon and diazoxon as substrates show normal distributions of enzyme activity ([34] and M. I. Mackness, unpublished observations).

These inter-individual and inter-ethnic differences in serum 'A'-esterase activity may have profound effects on the toxicity of OP compounds to individuals or populations at risk of exposure either accidentally or through work [34, 38]. In industry, it may be advisable to establish which individuals are most at risk. It would appear sensible to ensure that those individuals with low activity are not exposed to high levels of OPs. Inter-individual differences in enzyme activity may have even greater relevance to soldiers and civilians exposed to OP nerve gases in time of war. Much more research will be needed before the effects of the enzyme polymorphism on detoxication capacity are fully understood.

Relationship with cystic fibrosis. Serum paraoxonase activity is genetically linked to cystic fibrosis (CF) in families afflicted with the disease [39, 40]. This finding opened the possibility of determining the gene responsible for cystic fibrosis by studying the enzyme at the molecular level. The recent discovery of restriction fragment length polymorphisms more closely linked to CF have made this intriguing possibility unlikely, although the study of the relationship between enzyme and disease may yet prove useful.

Link to atherosclerosis. In the context of preventive medicine, the possibility of using the serum enzyme(s) as an indicator for the development of atherosclerosis is of considerable interest. In the serum of humans, much of the activity measured with paraoxon as substrate (paraoxonase) is associated with HDL [11], and the activity of this enzyme is lower in the serum of patients who have suffered a myocardial infarct [31]. Premature coronary artery disease has been associated with low concentrations

Table 1. Paraoxonase activity in the serum HDL of controls and patients with 'Fish-eye' and Tangier disease

Subjects	HDL paraoxonase activity*
Control	58.4 ± 3.6
'Fish-eye' disease	5.5 ± 1.2
Tangier disease	0

* Activity = total activity in the lipoprotein fractions (nmol *p*-nitrophenol produced per min) and is the mean activity ±SD (N = 70 for controls; N = 2 for 'Fish-eye' disease).

of HDL [41, 42] and apolipoproteins A-I and A-II [43, 44] in plasma, indicating the possibility of a relationship between this esterase activity in the HDL and the amounts of apolipoproteins A-I or A-II or both found in the HDL.

'Fish-eye' disease (FED) is a familial condition characterized by severe corneal opacities and abnormal plasma lipoproteins [45, 46]. The HDL-cholesterol concentration in plasma is decreased by 90%, as are HDL apolipoproteins A-I and A-II. The HDL-'A'-esterase activity in these subjects is reduced by 89% compared to controls ([47] and Table 1). In a patient with Tangier disease, a condition of analphalipoproteinemia where the levels of apolipoprotein A-I and A-II are 100 and 10 times lower respectively than controls [48], no paraoxonase activity could be detected.

The results strengthen the evidence for a relationship between serum paraoxonase activity and HDL and its apolipoproteins (particularly apo A-I) and indicate the possibility of using the serum enzyme as an indicator of susceptibility to the development of atherosclerosis.

Future prospects for research. Perhaps the most pertinent question pertaining to future research on 'A'-esterases is, 'Is the serum enzyme predictive of heart disease?'. This possibility has been strengthened by the recent publication of a study conducted in Hungary. This investigation found that the children of patients who had suffered an early infarct had much lower levels of serum paraoxonase activity than control children of parents who had not had an infarct and concluded that low 'A'-esterase activity may be connected with the possibility of premature myocardial infarction [49]. The proper biochemical characterisation of the enzyme(s) present in human serum may answer the question of whether the enzymes play a role in lipid metabolism, the perturbation of which, through low (possibly genetically determined) activity, can lead to the arterial deposition of lipids characteristic of atherosclerosis and thus indicate whether the enzyme can be used as a predictive tool. Mammalian serum 'A'-esterases have, as yet, no known *in vivo* function other than the detoxication of OPs. Rat liver microsomal 'B'-esterases hydrolyse palmitoyl-CoA and monoacylglycerides as well as various ester- and amide-type drugs [50–52]. In rabbits the presence or absence of a particular esterase band after electrophoresis seems to be linked with the ability of a particular strain to cope with a high cholesterol diet [53], indicating that an esterase may play a role in controlling cholesterol levels in the blood by metabolising cholesterol esters. In addition, the reaction catalysed by 'A'-esterases is similar to the hydrolytic action of phospholipases C and D. Possibly 'A'-esterases also have a function in lipid metabolism. Their location in HDL is consistent with such a role. However, at present, there is no known natural substrate for 'A'-esterase, and this area should also provide wide scope for future research.

The neglect of serum 'A'-esterases is surprising, both from the points of view of applied science (toxicology and medicine) and pure science. Applied aspects have been reviewed, such as the interaction with organophosphates and the link to disease states. It is also quite fascinating that some very active esterases with the interesting property of hydrolysing lipophilic ester poisons are well represented in mammalian serum lipoproteins. If the importance of these enzymes in clinical science can be proven, they will not be neglected for long.

Note added in proof: The classification of 'A'-esterases was reviewed at the 'International Meeting on Esterases Hydrolysing Organophosphorus Compounds' held at the Inter-University Centre in Dubrovnik, Yugoslavia, April 1988. The meeting decided to recommend to the NC-IUB that the 'A'-esterases be removed from classification EC 3.1.1.2 (Arylesterase) and together with DFPase (EC 3.8.2.1) form a new classification group to be called 'Organophosphorus Compound Hydrolase'.

Acknowledgements—The author would like to thank Mr. C. H. Walker for useful discussion during the preparation of this manuscript and Mrs. S. Dalton for typing the manuscript. Serum from Tangier patient was kindly supplied by M. Clerc. This work was supported by the Medical Research Council of Great Britain.

REFERENCES

1. Aldridge WN, Serum esterases. 1. Two types of esterase (A and B) hydrolysing *p*-nitrophenyl acetate, propionate and butyrate, and a method for their determination. *Biochem J* **53**: 110–117, 1953.
2. Aldridge WN, Serum esterases, 2. An enzyme hydrolysing diethyl p-nitrophenyl phosphate (E600) and its identity with the A-esterase of mammalian sera. *Biochem J* **53**: 117–124 1953.
3. NC-IUB, *Enzyme Nomenclature 1984*, pp. 271–278. Academic Press, Orlando 1984.
4. Mackness MI, Thompson HM, Hardy AR and Walker CH, Distinction between 'A'-esterases and arylesterases. *Biochem J* **245**: 293–296, 1987.
5. Chemnitius J-M, Losch H, Losch K and Zech R, Organophosphate detoxicating hydrolases in different vertebrate species. *Comp Biochem Physiol* **76C**: 85–93, 1983.
6. Shishido T and Fukami J-I, Enzymatic hydrolysis of diazoxon by rat tissue homogenates. *Pestic Biochem Physiol* **2**: 39–50. 1972.
7. Mackness MI and Walker CH. 'A'-esterase activity in the lipoprotein fraction of sheep serum. *Biochem Pharmacol* **30**: 903–906, 1981.
8. Eto M, *Organophosphorus Pesticides: Organic and Biological Chemistry*. CRC Press, Cleveland, OH 1974.
9. O'Brien RD, *Insecticide Action and Metabolism*. Academic Press, New York, 1967.
10. Mackness MI and Walker CH, Partial purification and properties of sheep serum 'A'-esterases. *Biochem Pharmacol* **32**: 2291–2296, 1983.

11. Mackness MI, Hallam SD, Peard T, Warner S and Walker CH, The separation of sheep and human serum 'A'-esterase activity into the lipoprotein fraction by ultracentrifugation. *Comp Biochem Physiol* **82B**: 675–677, 1985.
12. Mackness MI, Hallam SD and Walker CH, 'A'-esterase activity in the lipoprotein fraction of sheep and human serum. *Biochem Soc Trans* **13**: 135–136, 1985.
13. Tanford C, *The Hydrophobic Effect*, 2nd Ed. Wiley-Interscience, New York, 1980.
14. Walker CH and Mackness MI, 'A'-esterases and their role in regulating the toxicity of organophosphates. *Arch Toxicol* **60**: 30–33, 1987.
15. Main AR, The role of 'A'-esterase in the acute toxicity of paraoxon, TEPP, and parathion. *Can J Biochem Physiol* **34**: 197–216, 1956.
16. Brealey CJ, Comparative metabolism of pirimiphos-methyl in rat and Japanese quail. *Ph.D. Thesis*. University of Reading, 1981.
17. Machin AF, Rogers H, Cross AJ, Quick MP, Howells LC and Janes NF, Metabolic aspects of the toxicology of diazinon 1. *Pestic Sci* **6**: 461–473, 1975.
18. Machin AF, Anderson PH, Quick MP, Waddell DF, Skibniewska KA and Howells LC, The metabolism of diazinon in the liver and blood of species of varying susceptibility to diazinon poisoning. *Xenobiotica* **7**: 104–105, 1978.
19. Brealey CJ, Walker CH and Baldwin BC, 'A'-esterase activities in relation to the differential toxicity of pirimiphos-methyl to birds and mammals. *Pestic Sci* **11**: 546–554, 1980.
20. Mackness MI, Walker CH, Rowlands DG and Price NR, Esterase activity in homogenates of three strains of the rust red flour beetle *Tribolium castaneum* (Herbst). *Comp Biochem Physiol* **74C**: 65–68, 1983.
21. Devonshire AL, The properties of a carboxylesterase from the peach-potato aphid (*Myzus persicae*) and its role in conferring insecticide resistance. *Biochem J* **167**: 675–683, 1977.
22. Devonshire AL and Moores GD, A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae*). *Pestic Biochem Physiol* **18**: 235–246, 1982.
23. Motoyama N, Kao LR, Lin PT and Dauterman WC, Dual role of esterases in insecticide resistance in the green rice leafhopper. *Pestic Biochem Physiol* **21**: 139–147, 1984.
24. Simpson NE, Serum arylesterase activity in twins and their parents. *Am J Hum Genet* **23**: 375–382, 1971.
25. Lorentz K, Flatter B and Augustin E, Arylesterase in serum: Elaboration and clinical application of a fixed-incubation method. *Clin Chem* **25**: 1714–1720, 1979.
26. Krisch K, Enzymatische hydrolyse von diathyl-*p*-nitrophenal phosphat (E600) durch menschliches serum. *Z Klin Chem Klin Biochem* **1**: 41–45, 1968.
27. Playfer JR, Eze LC, Bullen MF and Evans DAP, Genetic polymorphism and interethnic variability of plasma paraoxonase activity. *J Med Genet* **13**: 337–342, 1976.
28. Carro-Ciampi G, Kadar D and Kalow W, Distribution of serum paraoxon hydrolysing activities in a Canadian population. *Can J Physiol Pharmacol* **59**: 904–907, 1981.
29. Eiberg H and Mohr J, Genetics of paraoxonase. *Ann Hum Genet* **45**: 323–330, 1981.
30. Eckerson MW, Romson J, Wyte C and La Du BN, The human serum paraoxonase polymorphism. *Am J Hum Genet* **35**: 214–227, 1983.
31. McElveen J, Mackness MI, Colley CM, Peard T, Warner S and Walker CH, Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. *Clin Chem* **32**: 671–673, 1986.
32. Flugel M and Geldmacher-von Mallinckrodt M, Zur kinetik des paraoxon spaltenden enzymes in menschlichen serum (EC 3.1.1.2). *Klin Wochenschr* **56**: 911–916, 1978.
33. Geldmacher-von Mallinckrodt M, Hommel G and Dumbach J, On the genetics of human serum paraoxonase. *Hum Genet* **52**: 313–326, 1979.
34. Geldmacher-von Mallinckrodt M, Diepgen TL and Enders PW, Interethnic differences in paraoxonase activity–Relevance for the detoxification of organophosphorus compounds. *Arch Belges Med Soc Hyg Med Travail Med Legale* (Suppl): 243–251, 1984.
35. Geldmacher-von Mallinckrodt M, Diepgen TL, Duhme C and Hommel G, A study of the polymorphism and ethnic distribution differences of human serum paraoxonase. *Am J Phys Anthropol* **62**: 235–241, 1983.
36. Carro-Ciampi G, Gray S and Kalow W, Paraoxonase phenotype distribution in Canadian Indian and Inuit populations. *Can J Physiol Pharmacol* **61**: 336–340 1983.
37. Diepgen TL and Geldmacher-von Mallinckrodt M, Interethnic differences in the detoxification of organophosphates. *Arch Toxicol* (Suppl 9): 154–158, 1986.
38. La Du BN and Eckerson HW, Could the human paraoxonase polymorphism account for different responses to certain environmental chemicals? In: *Genetic Variability in Responses in Chemical Exposure (Banbury Report No. 16)* (Ed. Omenn GS), pp. 167–175, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1984.
39. Eiberg H, Mohr J, Schmiegelow K, Nielsen LS and Williamson R, Linkage relationships of paraoxonase (PON) with other markers: Indication of PON-cystic fibrosis synteny. *Clin Genet* **28**: 265–271, 1985.
40. Schmiegelow K, Eiberg H, Tsui L-C, Buckwald M, Phelan PD, Williamson R, Warwick W, Niebuhr E, Mohr J, Schwartz M and Koch C, Linkage between the loci for cystic fibrosis and paraoxonase. *Clin Genet* **29**: 374–377, 1986.
41. Miller GJ and Miller NE, Plasma high density lipoprotein concentration and the development of ischaemic heart disease. *Lancet* **i**: 16–19, 1975.
42. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A and Zukel WJ, HDL cholesterol and other lipids in coronary heart disease. *Circulation* **55**: 767–772, 1977.
43. Maciejko JJ, Holmes DR, Kottke BA, Zinsmeister AR, Dinh DM and Mao SJT, Apolipoprotein A-I as a marker of angiographically assessed coronary-artery disease. *N Engl J Med* **309**: 385–389, 1983.
44. Albers JJ, Cheung MC and Hazzard WR, High density lipoproteins in myocardial infarction survivors. *Metabolism* **27**: 479–485, 1978.
45. Carlson LA and Philipson B, Fish-eye disease. A new familial condition with massive corneal opacities and dyslipoproteinaemia. *Lancet* **ii**: 921–923, 1979.
46. Carlson LA, Fish-eye disease: A new familial condition with massive corneal opacities and dyslipoproteinaemia. *Eur J Clin Invest* **12**: 41–53, 1982.
47. Mackness MI, Walker CH and Carlson LA, Low A-esterase activity in serum of patients with fish-eye disease. *Clin Chem* **33**: 587–588, 1987.
48. Dumon MF, Visvikis S, Manabe T and Clerc M, Immunochemical study of the plasma low and high density lipoproteins in Tangier disease. *FEBS Letts* **201**: 163–167, 1986.
49. Szabo I, Rona K, Czinner A, Gachalji B and Kaldor A, Is paraoxon hydrolytic activity in serum predictive of myocardial infarction? *Clin Chem* **33**: 742–743, 1987.
50. Mentlein R, Heiland S and Heymann E, Simultaneous purification and comparative characterisation of six

serine hydrolases from rat liver microsomes. *Arch Biochem Biophys* **200**: 547–559, 1980.
51. Mentlein R and Heymann E, Hydrolysis of ester- and amide-type drugs by purified isoenzymes of non-specific carboxylesterase from rat liver. *Biochemical Pharmacol* **33**: 1243–1248, 1984.
52. Mentlein R, Berge RK and Heymann E, Identity of purified monoacyglycerol lipase, palmitoyl-CoA hydrolase and aspirin-metabolizing carboxylesterase from rat liver microsomal fractions. *Biochem J* **232**: 479–483, 1985.
53. Van Zutphen LFM and Fox RR, Strain differences in response to dietary cholesterol by JAX rabbits: Correlation with esterase patterns. *Atherosclerosis* **28**: 435–446, 1977.