THE EFFECT OF TEMPERATURE ON
FLUORIDE-RESISTANT SERUM CHOLINESTERASE

BY
J. KING, M. J. MCQUEEN AND H. G. MORGAN

SUMMARY
Two individuals homozygous for the fluoride-resistant serum cholinesterase have been found in two generations of one family. A study of the variation with temperature of serum cholinesterase activity and the effect of inhibitors confirms earlier theoretical predictions.

By means of dibucaine inhibition (Kalow and Genest, 1957) it was found possible to differentiate three inherited serum cholinesterase phenotypes, those homozygous for the usual enzyme, homozygotes of the atypical enzyme and heterozygous individuals (Kalow and Staron, 1957). Harris and Whittaker (1961) then showed that sodium fluoride could be used to differentiate the serum cholinesterase variants in a manner similar to that of dibucaine. In a study of suxamethonium-sensitive patients and their families, Harris and Whittaker (1961) found that fluoride inhibition revealed two further groups. Later studies (Harris and Whittaker, 1962, 1963) showed that these were the results of another allelic gene which was termed fluoride-resistant. Lehmann and associates (1963) first reported the existence of the phenotype homozygous for the fluoride-resistant gene. The occurrence of three further cases has been reported (Liddell, Lehmann and Davies, 1963; Whittaker, 1964; Griffiths, Davies and Lehmann, 1966). Lehmann and Liddell (1969) have estimated that the fluoride-resistant homozygote occurs once in 300,000 of the population and thus is the rarest of the cholinesterase variants.

The effect of the reaction temperature on the behaviour of the serum cholinesterases has been reported previously (King and Dixon, 1969, 1970; King and Morgan, 1970). In these studies it was noted that the presence of a silent gene (Liddell, Lehmann and Silk, 1962) made for homozygous character with regard to temperature effects as it does with inhibitor numbers. The temperature-activity relationships of six genotypes covering the behaviour of four phenotypes were given in the first report (King and Dixon, 1969). The usual/fluoride-resistant heterozygote and the fluoride-resistant homozygote were not available for study. In the description of the effect of temperature on the inhibitor numbers of serum cholinesterase (King and Dixon, 1970) no data regarding the fluoride-resistant homozygote could be given. The present report rectifies this.

METHODS
Following the scheme of King (1965) serum cholinesterase activity was estimated by the method of Kalow and Lindsay (1955) simultaneously with inhibition by 10 μM dibucaine (Kalow and Genest, 1957), 50 μM sodium fluoride (Harris and Whittaker, 1961) and 500 mM sodium chloride (Whittaker, 1968) in a Unicam SP8000 recording spectrophotometer with temperature control given by a Tecam water-bath and dip-cooler.

The convention proposed by Motulsky (1964) for describing the genetic variants of the first cholinesterase locus is used throughout.

Case report.
The patient, J. B., suffered prolonged paralysis following suxamethonium administration prior to external cephalic version of her second child. Assay of her serum at this time yielded the usual anomalous figures associated with late pregnancy but later results indicated a phenotype of £,£,£ with cholinesterase activity of 480 mU/ml at

J. KING, B.SC., M.I.BIOL., L.R.L.C., F.I.M.L.T., Department of Biochemistry; M. J. MCQUEEN, M.B., CH.B., Cardiac Surgery Unit; Professor H. G. MORGAN, B.SC., M.B., CH.B., F.R.C.P. (EDINB. & GLASG.), F.R.C.PATH., F.R.S.E., Department of Pathological Biochemistry; Royal Infirmary, Glasgow.
25°C, dibucaine, fluoride and chloride numbers of 64, 36 and 28 respectively. The baby girl was found to be genotype $E^e E^f$ as was the elder boy whose serum cholinesterase activity was 1010 mU/ml at 25°C with DN=70, FN=47 and CN=19. Assay of the husband’s serum gave results for cholinesterase activity of 695 mU/ml at 25°C and DN=79, FN=59 and CN=10, a normal phenotype although the lowish activity in a healthy young adult might suggest the presence of a silent gene. Equally, of course, the patient’s phenotypic behaviour might mask a silent gene (Whittaker, 1967; Simpson, 1967).

The patient’s mother was deceased but her father with dibucaine, fluoride and chloride numbers respectively of 74, 52 and 18 and activity of 645 mU/ml at 25°C was classified as a usual/fluoride-resistant heterozygote, $E^e E^f$. The paternal uncle was found to be a usual homozygote $E^e E^e$, and the paternal aunt, $E^e E^f$. On examining the maternal uncle and aunt it was found that they were homozygous to the usual and fluoride-resistant genes respectively. The aunt had a serum activity of 780 mU/ml at 25°C, DN=67, FN=39 and CN=26, a second fluoride-resistant homozygote in the same family but in an earlier generation. This evidence of the presence of a fluoride-resistant gene in the maternal family made it unnecessary to postulate the presence of a silent gene and the serum cholinesterase activity of 1400 mU/ml in the maternal uncle made it improbable. The cholinesterase phenotyping of this family is shown in figure 1.

**DISCUSSION**

The temperature-activity relationship of the patient (A), her husband (B), her son (C), father (D) and maternal aunt (E) are shown in figure 2. As predicted (King and Dixon, 1969) the fluoride-resistant homozygote exhibits anomalous behaviour “similar to, but not so pronounced as the atypical/fluoride-resistant heterozygote”. This latter genotype was shown to have an activity maximum at 36–37°C while the atypical homozygotes exhibited peaks at 32–34°C. From the curves A and E of figure 2 it is seen that above 40°C there is a progressive inactivation of the serum activity of phenotype $E^e E^f$. Further it has been confirmed that, as with other variants, this is a reversible thermal inactivation, not a denaturation.
heterozygote $E^E_E^f$ as shown by the curves C and D (fig. 2) which exhibit maximum activity at 48–49°C is in keeping with the lack of sensitivity to suxamethonium of such individuals.

Figure 3 shows the variation with temperature of the inhibitor numbers of cholinesterase phenotype $E^E_E^f$. As with other variants it is found that the chloride number is little affected by temperature, that the dibucaine number decreases rapidly at higher temperatures while fluoride inhibition is extremely sensitive to change in reaction temperature and care must be taken to keep this constant for meaningful results to be obtained. Indeed without thermostatic control, serum from an atypical heterozygote $E^E_E^f$ would yield figures at 30°C which would suggest the phenotype $E^fE^f$. Equally at 20°C the fluoride-resistant homozygote would give values for inhibitor numbers which could lead to a classification of $E^fE^f$ or $E^fE^f$. It is even possible that the rarity of the phenotype $E^fE^f$ may be due to a failure to appreciate this need for strict temperature control.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr M. D. Black for bringing this case to their attention and to all members of the family concerned for their ready co-operation.

REFERENCES


L'EFFET DE LA TEMPERATURE SUR LA CHOLINESTERASE SERIQUE, RESISTANTE A LA FLUORIDE

SOMMAIRE
Deux personnes homozygotes pour la cholinesterase sérique, résistante à la fluoride, ont été trouvées dans deux générations d'une famille. Une étude de la variation sous l'effet de la température de l'activité de la cholinesterase sérique et l'action des inhibiteurs confirmant les prédictions théoriques antérieures.

TEMPERATUREEFFEKTE AUF FLUORID-RESISTENTE SERUM-CHOLINESTERASE

ZUSAMMENFASSUNG
In zwei Generationen einer Familie wurden zwei Individuen ermittelt, die für die fluorid-resistente Cholinesterase homozygot waren. Eine Untersuchung der Veränderungen der Serum-Cholinesterase-Aktivitäten mit der Temperatur und der Wirkung von Inhibitoren bestätigt frühere theoretische Vermutungen.

EL EFECTO DE LA TEMPERATURA SOBRE LA COLINESTERASA SERICA RESISTENTE AL FLUORURO

RESUMEN
Han sido hallados dos individuos en dos generaciones de una familia que son homocigotos para la colinesterasa sérica resistente al fluoruro. Un estudio de la variación de la actividad de la colinesterasa sérica con la temperatura y del efecto de inhibidores confirma las predicciones teóricas previas.

---

THE SOCIETY OF ANAESTHETISTS OF WALES

Programme for 1971–72

OCTOBER 23, 1971
Annual Dinner. Guest Speaker: Mr Per Saugman (Blackwell Scientific Publications, Oxford).

JANUARY 8, 1972
Clinico-Scientific Meeting: Professor J. W. Dundee (Belfast).

JUNE 24, 1972
Summer Clinical Meeting in Cambridge (by invitation of the East Anglian Association of Anaesthetists).

Full details of the above may be obtained from the Hon. Secretary, Society of Anaesthetists of Wales, c/o Department of Anaesthetics, University Hospital of Wales, Heath, Cardiff CF4 4XW.