Letters to the Editor

PHENYLALANINE HYDROXYLATION IN CULTURED FIBROBLASTS FROM PATIENTS WITH PHENYLKETONURIA

SIR,—Estimation of the activity of phenylalanine hydroxylase in cultured human fibroblasts would permit characterisation of enzyme defects in variants of phenylketonuria (P.K.U.).[1-3] Furthermore, enzyme activity might also be detectable in cultured human amniotic cells, yielding a prenatal test for P.K.U. Differences in hydroxylation-rate between normal individuals and patients with P.K.U. should highlight differences in the expression of the gene coding for phenylalanine hydroxylase in human fibroblasts. We have found that the oxidation of phenylalanine to tyrosine in fibroblastic proteins from controls and patients with P.K.U. is in fact different. (The diagnosis of P.K.U. is based on clinical data only. Phenylalanine levels in serum exceeded 20 mg/dl, but further characterisation by loading tests[3] has not been done.)

Enzyme activity was tested in homogenates of fibroblasts cultured by standard methods. The reaction mixture (final volume 0·15 ml) contained the following components: 0·15 mg protein of homogenised fibroblasts, centrifuged at 45 000*g* for 30 min, "tris" buffer pH 7·44 75 mmol/l, magnesium chloride 3·0 mmol/l, D,L 5,6,7,8-tetrahydrobiopterin dihydrochloride 19 μmol/l (a gift from Dr W. Kapp and Dr S. Skunca, Hoffmann-La Roche, Grenzach-Wyhlen), catalase 0·03 mg, N.A.D.H 0·49 mmol/l, ^{14}C-phenylalanine 0·8 μCi/assay, specific activity 486 mCi/mmol (Amersham Buchler), ^{12}C+^{14}C phenylalanine 26 μmol/l. Incubation was performed at 37°C for 90 min. After chromatographic separation of ^{14}C-phenylalanine from its reaction products and liquid scintillation counting enzyme activity was determined by the amount of ^{14}C-tyrosine formed.

FORMATION OF TYROSINE FROM L-PHENYLALANINE BY PROTEINS FROM HUMAN FIBROBLASTS OF NORMAL PERSONS AND OF PATIENTS CLINICALLY DIAGNOSED AS CLASSICAL P.K.U.

Group	No. of determinations	Tyrosine formed (pmol/h/mg) (mean ± S.D.)
10 controls	15	45·0 ± 4·5
P.K.U. ·	15	8·0 ± 6·9
A	6	0·0 ± 1·8
B	3	7·0 ± 5·7
C	3	8·1 ± 4·7
D	3	16·9 ± 6·0

We have compared the proteins of cultured fibroblasts of ten normal individuals of unknown state of heterozygosity matched in age and sex to four fibroblastic strains of patients with P.K.U. As can be seen from the table we observed a mean conversion of phenylalanine to tyrosine (given in pmol/h/mg fibroblastic protein) of 45·0 by normal individuals and 8·0 by patients with P.K.U.

Although three of the four patients oxidise a considerable amount of phenylalanine there is no overlapping in enzymatic activity within the two groups, at least in the range up to the third standard deviation. This result demonstrates in accordance with the values of the standard deviations obtained by testing fibroblastic strains of the same person for several times that P.K.U. can be diagnosed in cultured human fibroblasts.

Further studies will be pursued with incubation of the fibroblasts of these patients with tetrahydropterins as Milstien and Kaufman[4] observed a stimulation of enzyme activity in liver slices by this procedure. Thus cultured human fibroblasts will be an excellent system for testing therapeutic possibilities for patients with P.K.U.

In all cultured fibroblasts *Mycoplasma* infection was excluded.[5]

A detailed description of kinetic characteristics of the phenylalanine hydroxylase in normal individuals compared with patients with P.K.U. is in preparation

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VINYL CHLORIDE AND MORTALITY?

SIR,—Excess cancer after exposure to vinyl chloride (V.C.) was demonstrated in animals by Viola et al.[6] and Maltoni and Lefemine[7] in Italy, and subsequently suggested by Monson et al.[8] and later definitively demonstrated by several investigations in man in the United States.[9-11] However, Duck et al.[12] of British Petroleum in the U.K. found no excess of cancer mortality—indeed, the longer workers were exposed to V.C., the healthier they seemed to be, as suggested by table II of their report, which shows a decreasing risk of death with an increasing duration of exposure.

In those exposed for less than 10 years, the standardised mortality from all causes was 112, but it fell to 107 for those exposed between 10 and 14 years and to 61 for those exposed for more than 15 years. Several interpretations of these findings are possible: (1) the formulated V.C. as received by B.P. is uniquely non-toxic, (2) V.C. as polymerised or processed at B.P. is uniquely safe, (3) workers at B.P. have a particular genetic endowment which decreases their likelihood for V.C.-induced cancer, or conversely, other working populations[3-6] have a unique susceptibility to V.C., or (4) certain dietary factors unique to the workers at B.P. may scavenge free-radical V.C. (e.g., some have advocated eating lots of onions or garlic containing free sulphydryl groups.[13]) Before venturing any interpretation in biological, occupational, or technological terms, however, a closer consideration of the B.P. data seems wise, especially in view of studies[14 15] which demonstrated that the S.M.R. for total mortality increases with an increased duration of employment, due to elimination of the "healthy worker" effect. If in a follow-up study one selects, for example, a subgroup of workers by the fact that they have achieved at least 15 years' exposure, then none of these workers could have died before the 15th anniversary, so information on risk of dying can only come from the number of man-years at risk and the number of deaths after 15 years. Of course these same men, provided they are properly regrouped together with those dying or coming to the end of the follow-up between, for example, 10 and 14 years can provide similar information for this time-interval—and so on for all previous time-intervals. This

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REANALYSIS OF DATA BY DUCK ET AL. SHOWING PREVIOUSLY REPORTED VERSUS ESTIMATED NUMBERS OF EXPECTED DEATHS AND S.M.R.'S BY DURATION OF EXPOSURE AND CAUSE OF DEATH

Duration of exposure yr	Cause of death									
	All causes					Total cancers				
	O	E		S.M.R.		O	E		S.M.R.	
		Duck et al.	RE	Duck et al.	RE		Duck et al	RE	Duck et al.	RE
<10	83	74·01	105·46	112	79	23	18·68	26·62	123	86
10–14	28	26·91	20·49	107	137	4	6·87	5·23	58	76
15+	25	41·30	7·09	61	353	8	10·89	1·87	73	428
	Digestive system cancer					Lung cancer				
<10	6	5·64	3·04	106	75	10	7·76	11·06	129	90
10–14	1	2·13	1·62	47	62	3	2·97	2·26	101	133
15+	4	3·31	0·57	121	702	3	4·80	0·82	62	366

O=Observed E=Expected. RE=Recalculated estimates.

is the principle of the life-table method, which Duck et al. seem to have ignored.

Their table II puts the man-years at risk for the 336 workers who had 15+ years duration of exposure at 6084. Our maximum estimate for this same category is only 4032. We assumed that all 336 workers began employment on Jan. 1, 1948 (the first day of the study period) and had continuous employment until Jan. 1, 1975, and that the 25 deaths in this group occurred on Jan. 1, 1975, thus estimating maximum man-years at risk. Since these workers belonged to the exposure group that completed 15+ years of employment, they would begin to accumulate man-years at risk of dying on Jan. 1, 1963, so by Jan. 1, 1975, the maximum estimate for man-years at risk is 336 men × 12 years of observation (=4032). Duck et al. would seem to have started accumulating man-years of observations for these workers immediately when employment began rather than after 15 years of employment, thus diluting the estimate of the risk of death after 15 years of exposure. We have redistributed the man-years at risk shown in table II of the original paper and recalculated the expected numbers of deaths, and the S.M.R.s. These data are shown in the accompanying table, alongside those reported in the original paper.

The 336 workers in the 15+ years exposure category had passed through the <10 and the 10–14 years exposure categories so we calculated the man-years at risk for the 15+ years exposure category by subtracting 336×10 years (<10 year period) and 336×5 years (10–14 years period) from 6084 to give 1044. The 3360 and 1680 man-years were then redistributed to the <10 year and 10–14 year exposure categories, respectively, and the process was repeated for the 10–14 year exposure category, and these excess years were also redistributed back to the <10 year exposure category. We then calculated the ratio of our man-years to those reported by Duck et al. and applied these ratios to the expected death figures in their report. For example, in the 15+ year exposure category, our estimate for total expected deaths is: 1044/6084 (=0·1716) × 41·30 (expected deaths reported by Duck et al.) = 7·09 (our expected). As shown in the table, there were (25 deaths observed/7·09) × 100=353 as our S.M.R. This is contrasted with an S.M.R. of 61, as reported by Duck et al. These same procedures were carried out for other causes of death using ratios of 0·7615 and 1·4294 for the 10–14 years and <10 years duration of exposure categories, respectively. Since the exact distribution for worker exposure is unknown to us, we recognise that our estimates only approximate the numbers of man-years at risk. However, conditional to this approximation, and contrary to what was stated by Duck et al.[12] ("there was no suggestion of an increased frequency of deaths from the most common malignant diseases"), a definite pattern of excessive site-specific cancer mortality associated with increasing duration of V.C. exposure clearly emerges.

As shown in the table and as expected from previous reports,[9 10 14 15] the S.M.R.'s for total mortality and cancer deaths tend to increase with an increased duration of occupational exposure. This example of misallocation of person-years at risk, the resultant erroneous conclusions, and subsequent reference to these conclusions[16 17] demonstrate the need for standardisation of data-handling techniques for cohort mortality studies, as suggested during a workshop sponsored by the International Agency for Research on Cancer in January, 1975, in Lyon, France.[18]

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*** This letter has been shown to the B.P. workers, whose reply follows.—ED. L.

SIR,—Dr Joan Davis (personal communication) has also pointed out the value of an additional tabulation of deaths more than fifteen years after first exposure in those exposed for at least fifteen years, and this has been calculated. We have just received these results (see table). The only major discrepancy between observed and expected deaths is in cancers of the digestive system. Two of these deaths were from stomach cancer and two from carcinoma of the colon. No firm conclusions can be drawn from such a small amount of data, and only the passage of time will establish whether there is any real excess of cancer mortality in this group.

MORTALITY OCCURRING AFTER FIFTEEN YEARS' EXPOSURE

—	Observed	Expected	S.M.R.
All causes	25	24·15	104
All cancer	8	6·51	123
Digestive system cancer	4	1·98	(202)
Lung cancer	3	2·96	(101)

We would like to point out the methodological errors which led Wagoner et al. to their gross underestimates of expected deaths. An age-standardised death-rate can be calculated only if the age structure of the population is known. The simplistic application of rates derived from a young population to one which is older—as a consequence of excluding the first fifteen years of exposure—will produce a considerable underestimate of expected deaths and hence inflate any mortality ratios which are calculated in this manner. The number of man-years in the long-exposure group will also be in excess of the presumed figure, since exposure records started in 1948, but death-rates have been studied for the period 1955–75. This design makes a life-table analysis for the whole study population impossible.

We investigated mortality during the period 1955–75 for three groups of workers in which each individual was classified by his total duration of exposure. Inevitably the low mortality throughout this period for the men with over fifteen years' exposure will reflect the long period of survival required to enter this group.

A mortality study with a detailed cohort analysis covering the whole U.K. polyvinyl chloride production industry is to be published shortly and the results will certainly be of interest.[19 20]

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