similar associations between other influenza epidemics and childhood leukaemia.

If the correlation shown by Fedrick and Alberman (1972) and supported by Bithell *et al.* (1973) and our study reflects a true causal relationship it is certainly premature to attribute this to the virus. We have previously emphasized the possible significance of heavy medication, correlating with influenza and teratogenesis (Hakosalo and Saxén, 1971; Karkinen-Jääskeläinen and Saxén, 1973), and there could be carcinogens among these drugs. On the other hand, it is then difficult to explain the uniqueness of the 1957 epidemic caused by the A₂ virus on this basis.

However, present knowledge of the variation of influenza A viruses shows that a profound alteration in the properties of the haemagglutinating and neuraminidase subunits of the influenza virion surface (Webster and Laver, 1972) occurs once in 10 to 30 years. This happened in 1957.

The alteration is characterized by a new antigenicity of both subunits. When the so-called Hong Kong variant appeared in 1969 analysis of tryptic peptides of the haemagglutinating proteins showed extensive alterations in its amino-acid sequence, indicating that substitution in the molecules had occurred (Webster and Laver, 1972). This is thought to be brought about by recombination within the genomes of different influenza viruses from different animal species, particularly avian and swine strains. New genetic information, unrelated to the influenza genome, may be transferred by recombinant strains to the human population. In some cases this information may traverse the placental barrier and reach the developing fetus, though viraemia in influenza and transfer through the placenta is thought to be rare (Monif *et al.*, 1973).

The appearance of a "new" influenza virus against which few or no antibodies exist anywhere in the world results in a highly contagious pandemic and possibly increased severity of the disease and its consequences. The latter may be observed as an increased mortality among some age groups, increased production of congenital malformations, and an increased rate of pneumonia and encephalitic syndromes. Not all the factors have been studied but the possibility of increased leukaemia incidence in children born to mothers who have had influenza during pregnancy is of special interest.

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Blood Lead Levels in a Welsh Rural Community

W. H. BEASLEY, D. D. JONES, A. MEGIT, SYLVIA G. LUTKINS

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Summary

In a study of the blood lead levels of 626 healthy blood donors no differences were found between men and women, but there was a significant increase with age. Resident donors had a higher blood lead than students, and the level in residents increased with living in the Aberystwyth area up to about 20 years. The levels in the students did not increase with residence in Aberystwyth. No differences were found in the blood lead of donors living in different wards of Aberystwyth and none between the blood lead of donors living in the rural area and those in the town. Almost half of the local resident donors had a level above the "normal" range.

Department of Pathology, Bronglais General Hospital, Aberystwyth SY23 1ER

W. H. BEASLEY, M.D., F.R.C.PATH., Consultant Pathologist
 D. D. JONES, M.Sc., Principal Biochemist
 A. MEGIT, B.Sc., Biochemist

Department of Statistics, University College of Wales, Aberystwyth
 SYLVIA G. LUTKINS, B.Sc., M.Sc., Senior Research Officer

Introduction

The poisonous properties of lead in excessive amounts have long been recognized. Accidental ingestion, particularly in children, has produced serious disease. It was one of the first metals to be used by man and there has been extensive exploitation to obtain it. Present in air, food, and water, lead is inevitably absorbed by man. It is not an element which is necessary for human metabolism, but whether its presence in the body causes any ill effects in people not exposed to lead industrially is disputed. One of the points of dispute is its so-called "normal" range in blood.

Kehoe *et al.* (1935) in the U.S.A. reported a mean blood lead level of 58 µg/100 ml (S.D. 21 µg) in 71 medical students, and Kehoe *et al.* (1940) one of 25 µg/100 g (S.D. 5 µg) in 30 normal Americans and 30 normal Mexicans. Horiuchi and Takada (1954) found in 95% of 44 normal Japanese that the blood lead level was less than 31 µg/100 g and Patterson (1965), from the U.S.A., reported a range of 5-40 µg/100 ml. The U.S. Department of Health (1965) reported that in 2,342 people only 11 had a concentration exceeding 60 µg/100 ml. Goldwater and Hoover (1967) found a range of 15-40 µg/100 ml in blood lead levels of 829 people from 16 countries. They added that the levels in New Guinea aborigines were comparable to those in people living in industrialized countries. After an investigation of the blood lead levels of 689 people living in the U.S.A., South America, Africa, and New Guinea, Stopps (1968) reported a range of 13-23 µg/100 g. Kubota *et al.* (1968) found in 243 healthy Americans a range of 7.25-20.34 µg/100 ml, and the level of blood lead depended on where in the U.S.A. the subject lived.

A statement in the *British Medical Journal* (1968), intended for guidance in suspected inorganic lead poisoning, said that the "normal" blood lead, when there had been no occupational or abnormal exposure, was less than 40 µg/100 ml. If the level were between 40 and 80 µg/100 ml it showed "increased absorption resulting from occupational or abnormal exposure which is occupationally acceptable." Hammond (1969) found a range of 11-26 µg/100 g in 35 normal people in Philadelphia. A conference held in Amsterdam in November 1968 (Selander and Cramer, 1970) concluded that the upper limit for acceptable lead absorption was 70 µg/100 ml. In Milan Zurlo *et al.* (1970) found in 90% of 138 adults not occupationally exposed to lead that the level was less than 50 µg/100 ml and never exceeded 70 µg/100 ml. L. B. Tepper (personal communication, 1972) reported a range of 12-20.6 µg/100 g in 2,006 women from 11 American cities.

Methods

On four successive days at a blood donor session in Aberystwyth in October 1971 blood samples for lead estimation were obtained from 626 donors, of whom 333 were students from the University College of Wales, Aberystwyth, and 293 were residents from Aberystwyth Borough and Rural areas and other parts of North Cardiganshire. The donors were not statistically selected.

CHEMICAL METHOD

The method was essentially that of Einarsson and Lindstedt (1969) also quoted in a bulletin from Southern Analytical, Ltd. (Shandon Southern Instruments, Camberley, Surrey). Protein was precipitated from 10 ml of whole heparinized blood by the addition of 7.5 ml of a mixture of 5% (w/v) trichloroacetic acid (3 vols) and concentrated perchloric acid (1 vol) and the resulting mixture was allowed to stand for one hour and then centrifuged. The supernatant was decanted completely and to it 0.5 ml of 4% ammonium pyrrolidine dithiocarbamate was added and mixed. Then 2 ml of xylene were added and extraction carried out by shaking mechanically for 10 minutes. Xylene was used because greater sensitivity was obtained than when the aqueous supernatant was sprayed.

The tubes were centrifuged and the organic phase was sprayed in a Southern Analytical A3000 atomic absorption spectrophotometer with an acetylene/air flame and the absorption measured at 217 nm, at which there is greater sensitivity than at 283 nm (Elwell and Gridley, 1966). Standards containing 20, 40, 60, 80, 100, and 200 µg/100 ml were similarly treated and blank values were obtained by the extraction of 10 ml protein precipitating reagent. The instrument was set to zero with xylene. In recovery experiments the mean recovery of lead was 94.5%.

Results of Statistical Analysis

The first statistical analysis was to establish whether men and women differed significantly in their blood lead levels. With the statistical technique of covariance, which effectively adjusted the lead values for age, it was shown that there was no difference and thus the data could be combined.

It was decided to analyse separately the residents and students who are usually in Aberystwyth for about 30 weeks of the year, so that the students might provide a comparison with local residents (table I). Comparison of the age-adjusted mean blood lead level of the residents (37.9) and that of the students (32.1) showed the difference to be significant at the 0.1% level.

The correlations between blood lead level and each of the variables "age" and "duration of local residence" were obtained

TABLE II—Correlation Coefficients of Blood Lead, Age, and Duration of Residence in Students (n = 333) and Residents (n = 293)

	Age	Duration of Residence	Lead Level
<i>Students</i>			
Age	1.00	0.29	0.14
Duration of residence ..		1.00	0.07
Lead level			1.00
<i>Residents</i>			
Age	1.00	0.32	0.13
Duration of residence ..		1.00	0.16
Lead level			1.00

(table II). For the student donors the age effect was more important than the duration of local residence, the former being significant at the 5% level. Age was also significant in the resident donors at the 5% level, but duration of local residence was significant at the 1% level, indicating a greater association with blood lead. Clearly, age and duration of local residence were highly correlated, and, as expected from the earlier analyses, sex was an insignificant factor in both groups.

A detailed investigation of these associations was made by considering several multiple regression analyses with age, sex, and duration of local residence as explanatory variables (Lutkins *et al.*, 1972). Taken together these three variables accounted for only 11% of the total variation in the blood lead levels, which is an indication that there may well be other relevant factors.

The relation between age-adjusted blood lead levels and duration of local residence for the resident donors is shown in table III. A simple linear regression analysis of blood lead level on duration of local residence showed that there was a slight positive increase, with the slope significant at the 1% level (regression coefficient, $b = +0.14$). In view of this result, it was decided to investigate the effect of "year in college" on the blood lead of the students. After allowing for age differences,

TABLE I—Blood Lead Levels in µg/100 ml for 626 Blood Donors

	No. of Donors	Range	Mean Blood Lead Level ± S.E.	Standard Deviation	95% Confidence Limits for Mean Blood Lead Level		Mean Age in Years	Age-adjusted Mean Blood Lead Level	95% Confidence Limits for Age-adjusted Mean Blood Lead Level	
					Lower	Upper			Lower	Upper
Students:										
Males	181	15-71	31.30 ± 0.83	11.12	29.67	32.93	21.1	31.01	29.36	32.66
Females	152	16-71	31.25 ± 0.95	11.67	29.38	33.12	20.1	31.59	29.80	33.38
Total	333	15-71	31.28 ± 0.62	11.36	30.06	32.48	20.6	32.06	30.84	33.28
Residents:										
Males	181	16-77	39.29 ± 0.87	11.67	37.58	41.00	32.2	39.18	37.49	40.87
Females	112	17-75	37.92 ± 1.08	11.44	35.78	40.06	30.0	38.09	35.93	40.25
Total	293	16-77	38.76 ± 0.68	11.58	37.43	40.09	31.3	37.87	36.56	39.18

The male and female blood lead levels of the students have been adjusted to the average age 20.6 years using the student regression coefficient 0.60. For the residents the average age of 31.3 was used and the regression coefficient of 0.13. To compare the students with the residents their average blood lead levels have been adjusted to the overall average of 25.6 using the within groups overall coefficient of 0.16 mg/100 ml per year of age.

TABLE III—Blood Lead Levels, Adjusted for Age, and Grouped According to Years of Residence in Aberystwyth Area for Resident Donors

Duration of Local Residence in Years	No. of Donors	Age-adjusted Blood Lead Mean Levels ($\mu\text{g}/100\text{ ml}$) $\pm$ S.E.
0-4	82	35.73 $\pm$ 1.28
5-9	43	37.87 $\pm$ 1.74
10-14	26	39.83 $\pm$ 2.27
15-19	36	43.26 $\pm$ 1.92
20-24	46	39.14 $\pm$ 1.69
25-29	15	43.13 $\pm$ 2.93
30-34	22	35.44 $\pm$ 2.44
35-39	9	43.63 $\pm$ 3.79
at least 40	14	41.94 $\pm$ 3.31
Total	293	

there was no evidence of any increase in blood lead level with the number of years the student had been in college.

The next analysis was to determine if the place of residence in Aberystwyth had a relation with blood lead level. Aberystwyth Borough has a population of about 12,000 and has six electoral divisions. Therefore, the borough donors were classified according to the divisions in which they lived (table IV). In division 1 about three-quarters of the houses have been built since the 1939-45 war. Divisions 2, 3, and 4 consist of pre-1914 houses, and in divisions 5 and 6 the houses were almost all built before 1939. No significant differences were found in this analysis, nor was there a significant difference between the borough and rural district mean results.

The distribution of the blood lead levels of the donors into the so-called "normal" and "acceptable" ranges (*British Medical Journal*, 1968) are shown in table V. Altogether, 20.1% of the students and 44.4% of the resident donors were above the "normal" upper level.

TABLE IV—Blood Lead Levels Adjusted for Age and Grouped According to Electoral Divisions for Resident Donors

Electoral Division	No. of Donors	Age-adjusted Blood Lead Mean Levels $\pm$ S.E. ($\mu\text{g}/100\text{ ml}$)
1	40	39.07 $\pm$ 1.89
2	18	39.37 $\pm$ 2.82
3	13	41.93 $\pm$ 3.32
4	8	41.43 $\pm$ 4.23
5	18	40.87 $\pm$ 2.82
6	28	37.51 $\pm$ 2.26
All 6 wards	125	39.49 $\pm$ 1.05
Rural district	93	40.36 $\pm$ 1.21

TABLE V—Distribution of Blood Donors into "Normal" and "Acceptable" Levels

	"Normal" Blood Lead Level (0-40 $\mu\text{g}/100\text{ ml}$)	"Acceptable" Blood Lead Level (41-80 $\mu\text{g}/100\text{ ml}$)	Total
Donors:			
Students	266 (79.9%)	67 (20.1%)	333
Residents	163 (55.6%)	130 (44.4%)	293
Total	429 (68.5%)	197 (31.5%)	626

Discussion

Aberystwyth lies in an area in which lead has been mined since Roman times. John Speed (1676) wrote in his *Atlas of Wales*: "At the head of the Ystwyth are certain veins of lead, a merchandise of no means regard or wealth." Within about 15 miles of the town there were, between 1845 and 1928, almost a hundred lead mines, the scars of which are still visible (Lewis, 1967).

Occasional analyses of the water supply in houses in Aberystwyth Borough and Rural areas in recent years have shown a

total hardness of between 6 and 25 mg/l.; the pH values have varied from 6.9 to 8.5; and lead levels have been between nil and 0.11 mg/l. Thus the water is soft, has a pH generally within the World Health Organization range (7.0-8.5), and its lead content has not exceeded the World Health Organization limit for water in overnight contact with lead pipes. It does not appear, therefore, that the local water supply contains excessive lead. This is apparently confirmed by the fact that blood lead was not higher in donors from the old houses in the town when compared with that in donors from houses built since 1945. However, it should be added that an extensive study of the water supply in the area would be a useful investigation.

The population of the borough and rural district of Aberystwyth is about 24,000. It is not, therefore, likely that such a small population in an area of about 200 square miles (518 km²) would create a high road traffic density with its consequent air pollution from lead compounds from petrol. Nevertheless, the correlations clearly showed that duration of local residence was a significant factor in the higher level of blood lead found in the resident donors. The regression coefficient of blood lead on duration of local residence was + 0.14, which indicated that, on average, there was a slight increase of 0.14 $\mu\text{g}/100\text{ ml}$ for every year spent in this locality. However, the mean levels in table III showed that after 20 years' residence the increase in blood lead began to fall off. Two further analyses confirmed this finding. When students and residents up to the age of 25 years were compared the mean blood lead levels of the residents was significantly higher (at the 0.1% level). Comparison of lifetime residents and other residents who had moved into the district and lived here for part of their lives (this part could be large or small) showed that the age-adjusted blood lead mean level of the lifetime residents was just significantly higher at the 5% level.

Consideration of the water supply, the atmosphere, and the age of the houses appears to exclude them as causes for the higher levels of blood lead found in residents compared to the students. However, one other factor, which is a local one and which has to be considered, is the lead in the rocks and barren spoil heaps that may still be seen in this area. During the period of active lead mining fields adjacent to and downstream from the mines became polluted by material carried by air and water. Alloway (1969) analysed pasture soils in the Ystwyth and Rheidol valleys, both of which converge in Aberystwyth, and found abnormally high lead levels, and Alloway and Davies (1971), in their report on trace metal content of soils affected by base metal mining, stated that the Ystwyth Valley was the worst polluted in West and North Wales, and that the Rheidol Valley had also suffered because of lead mines. The same authors (1971) have also shown that plants grown on soils with enhanced metal concentrations have increased metal content, and that maximum acceptable lead concentrations were exceeded in apparently healthy crops.

Analysis by Abdullah *et al.* (1972) showed that the mean lead level in the sea water of Cardigan Bay was higher than that in Liverpool Bay or in the Bristol Channel and in both of these there is much more industrial and domestic effluent. This suggests that the run-off from the mineralized zones is the major source of this higher lead level. In a later report on heavy metals in river waters, Abdullah and Royle (1972) found lead levels in the rivers Ystwyth and Rheidol, which converge in Aberystwyth, to be two to eight times greater than that in "clean" streams. A survey by the Applied Geochemistry Research Group at the Imperial College of Science, London, (unpublished) of streams in the Aberystwyth area has shown high and very high levels of lead in the sediments.

These facts show an excess of lead in the river waters and streams. If lead pollution from petrol is excluded, and if the mains water supply seems unlikely to be an important source of lead, then the difference between the blood lead in students newly arrived in Aberystwyth and the local residents may be related to the lead in the area which is reflected in the high river and sea water levels. But in what way does this excess of lead get into the human body? Contamination of local pastures and

hence of food and milk may be a route, but the evidence suggests that most of the food sold here is imported into the Aberystwyth area. Nevertheless, analyses for air-borne lead may be worth making to determine if dust blown from old lead mine spoil heaps is a relevant factor.

No differences were found in this series between the blood lead levels of males and female donors. Hofreuter *et al.* (1961) and Thomas *et al.* (1967) reported higher levels in men than in women, and the former explained this on the grounds that men were more exposed to all sources of lead. Nevertheless, the report of the U.S. Department of Health (1965) thought that the relation between blood lead and sex was of "questionable significance." Goldwater and Hoover (1967) surveyed 16 countries and found no difference between the blood lead levels of males and females.

Several reports have shown no increase of blood lead with age (Hofreuter *et al.*, 1961; U.S. Department of Health, 1965; Horiuchi and Takada, 1964; Hammond, 1969). In this investigation an increase of blood lead with age was found in both the resident and student donors. The regression coefficient of blood lead on age was + 0.13 for residents and + 0.60 for students, both significant at the 5% level.

One other finding was the significant linear increase of blood lead among the resident donors according to the length of time they had resided locally, with an apparent tailing-off after about 20 years. If blood lead reflects the soft tissue concentration of lead then it appears, so far as this area is concerned, that there is not an increasing accumulation of lead in the soft tissues after 20 years of residence here. Schroeder and Tipton (1968) found accumulation of lead in soft tissues of American peoples up to the fifth or sixth decade of age and not thereafter, but found little or no accumulation of lead in tissues of non-Americans after the second decade. Barry and Mossman (1970) also found no increase of lead after the second decade of age in the soft tissues of persons in the North-West of England.

If the statement in the *British Medical Journal* (1968) is taken as a standard for adults then 56% of the local resident donors were in the so-called "normal" range of 0-40 µg/100 ml and 44% in the "acceptable" range 41-80 µg/100 ml. The corresponding figures for students were 80% in the normal and 20% in the acceptable range. All these donors stated that they were healthy, yet nearly half of the resident donors were above the "normal" upper limit.

Nevertheless, it is difficult to make a true comparison of blood lead levels in people from various parts of the world, or with "normal" and "acceptable" ranges, when there are variations

of technique that cause wide differences in the results. This is a problem that now needs urgent attention so that the hazard of lead to health can be more clearly defined.

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