# Lead surprise — in via the skin

We all know that lead is toxic — hence the shift to lead-free petrol, a move indicative of a growing concern about inadvertent poisoning by a widely used element.

Lead can cause changes in the synthesis of the oxygen-carrying, red-blood-cell pigment haemoglobin, giving rise to anaemia. At higher concentrations, it affects the kidneys, the nervous system (especially in children and babies), and reproduction (see the box on page 16).

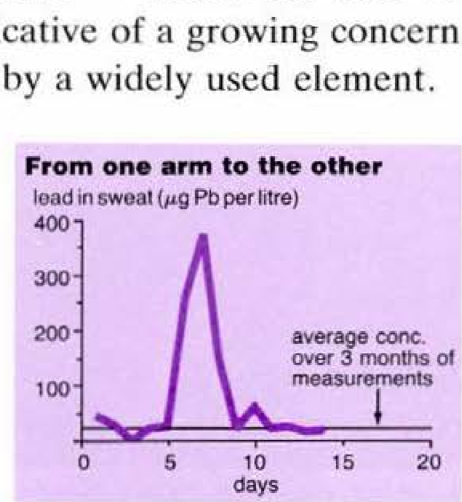
Obviously, health authorities and employers are generally aware of the dangers of lead in the environment. They have acted to reduce the risk of poisoning from eating food high in lead or from breathing in lead-rich dust. Less often considered is a third route of entry: the skin.

Unless you have a cut, your skin usually forms a fairly efficient barrier to the entry of chemicals and micro-organisms. But like any substance it has its limitations, and certain compounds, especially if they are fat-soluble, can pass across it. (That is how medicinal skin creams can get their active ingredient through the outer layer.)

Most fat-soluble substances are organic — that means, essentially, carbon-containing. Researchers in the 1940s and '50s showed that organic lead compounds, especially tetraethyl-lead, could pass across human skin and into the blood. However, inorganic compounds, such as lead oxide or lead nitrate, did not do so in those experiments. Therefore, in industries where workers had contact with inorganic lead — such as lead smelting or the manufacture of batteries — no great concern existed about levels of lead-rich dust on the skin, although precautions were certainly taken to prevent inhalation or ingestion.

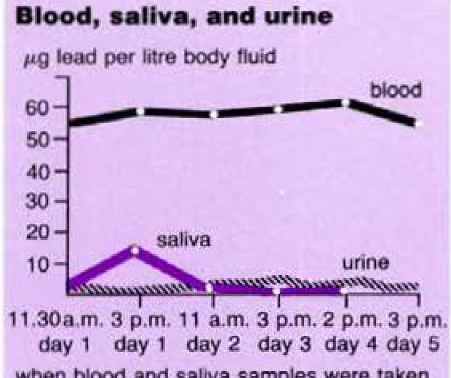
But now all this has been turned on its head, and some textbooks will be in need of revision following work by Dr Mark Florence, Mr Steve Lilley, and Ms Jenny Stauber at the CSIRO Division of Fuel Technology in Sydney. They have recently carried out a re-investigation of lead absorption through the skin, with a grant from the Australian National Occupational Health and Safety Commission. As far as they are aware, they are the only group in the world currently studying the absorption of inorganic metal compounds through the skin. Their recent findings have important implications for the health of workers in some industries.

**From one arm to the other**




**The graph shows the lead level in sweat from the right arm of a subject after scientists placed a lead nitrate solution on the skin of the left arm (on day 5 for 24 hours).**

**Blood, saliva, and urine**




**The lead level in saliva also jumped, but not that in blood or urine. (The lead nitrate solution was applied at noon on day 1 and remained on the skin of the left arm for 24 hours.)**

Any investigation of this nature in humans needs two essential techniques. The most important is a method of rapid and accurate detection of tiny concentrations of the substance in question within biological media; the second is an easy and quick means of obtaining representative samples of human tissues or fluid from volunteers.

Now, a scientist is more likely to find volunteers if his or her methods are painless and 'non-invasive'; also, and perhaps more importantly in the light of the increase in blood-borne communicable diseases, blood sampling is not always safe. It is therefore preferable, where possible, to analyse more readily collected and less dangerous offerings — such as saliva, tears, sweat, hair, and nails.

Dr Florence and his colleagues used a technique called anodic stripping voltammetry to detect minute traces of metals in the biological fluid they had chosen — sweat.

In this grandly named analytical technique, metal ions in solution (for example, lead) are reduced and deposited into an oppositely charged mercury electrode, forming an amalgam with it. (Obviously, the higher the metal concentration rises, the greater is the amount deposited in the electrode — provided we keep constant the time, on each occasion, that the electrode is exposed to the solution.) A positive potential sweep then strips the metal out of the electrode; and the current required to do this, and return the electrode to its original state, is thus directly proportional to the starting concentration of ions in solution.

For lead, this technique can measure concentrations as low as 2 ng per litre (a nanogram — ng — is a billionth of a gram). Dr Florence has also used anodic stripping voltammetry to analyse human sweat for zinc, copper, and cadmium, which can also form amalgams with the electrode; the technique is not effective for substances that cannot do so. We reveal what good honest sweat contains on page 16.

## Getting sweat

But how do you collect sweat without it becoming contaminated? Medical researchers analysing sweat have used various means ranging from the sublime — using saunas — to the ridiculous — enclosing subjects from the neck down in plastic bags in a hot room. A popular idea is keeping the forearm and fingers in a plastic glove and applying heat locally. However, all these methods involve the possibility of contamination if you are looking for tiny traces of metals.

So Dr Florence turned again to an electrical technique. He used iontophoresis — the passing of a small, constant electric current across the skin — to induce sweating. The current works in conjunction with a chemical, an alkaloid called pilocarpine, that activates the sweat glands. Two minutes of the passage of electric current forces pilocarpine into the sweat glands around the positive electrode; as a result, after removal of the electrodes, that area sweats for 30–60 minutes.

At the start of the procedure the scientists carefully wash the skin to remove any surface contaminants. They then apply a type of absorbent pad, carefully chosen to avoid contamination, to cover the sweaty area. When sweating has ceased, they remove the pad and weigh it to determine how much sweat accumulated — usually, about 0·05–0·07 g. They then analyse it.

Once Dr Florence had perfected this technique for collecting and analysing lead, zinc, copper, and cadmium in 'normal' sweat, he turned his attention to using sweat analysis as a means of seeing whether inorganic lead could cross the skin.

He put lead metal or lead salts on the skin of a volunteer and monitored the concentration of lead in sweat. The volunteer kept a lead-impregnated filter paper wrapped onto his arm with household clear plastic wrap for 6 hours a day for 4 days. Then the scientists took off the filter, and washed the relevant patch of skin with a chemical that removes lead.

### Lead moves

Before and after applying the lead, they took sweat samples from the area concerned and from the skin on the opposite arm. Their findings are intriguing. Lead concentrations in sweat increased enormously in the area exposed to the element, indicating that the inorganic form was finding its way into the sweat ducts. (Remember, after application of the lead, the scientists washed all traces off the skin.)

But also, the level of the metal increased in the sweat samples taken from the other arm. It did not do so nearly as much as on the arm exposed to lead, but it did rise quite obviously compared with the baseline reading before the experiment. This strongly suggested that lead was entering the body through the skin and in such an amount that its level in 'normal' sweat — as measured on the other arm — increased.

For example, after just 3 hours exposure of the left arm to lead, the lead in sweat from the right arm increased from 15 μg per litre to 115 μg per litre. This suggests that the metal can be absorbed through the skin and enter the blood-stream to be transported around the body.

Dr Florence believes that the major route for this lead transport across the skin is via the sweat ducts. He knows that inorganic lead diffuses almost a million times faster in sweat than through the material of dry skin. In the experiment, the patch of skin exposed to it was wet with sweat and the sweat ducts would have been filled with fluid through which the lead could move. Once in the sweat ducts, the element could cross their thin walls into the blood capillaries.

Dr Florence and his colleagues also monitored lead in blood, urine, and saliva as a further check on the lead levels in their volunteers' bodies. Strangely enough, they found that the concentrations in urine and blood did not increase significantly after they had put lead on the skin of the arm. But the quantity of lead in saliva did, jumping from 4 to 14 μg per litre about 3 hours after the patch went on the skin — about the same time that the highest lead value in sweat from the other arm occurred.

A likely explanation for this apparent anomaly is as follows: lead is tranported in the plasma (the fluid part of blood), but moves so quickly out of it into what biologists term the extracellular fluid pool that its residence in plasma is very brief. Consequently little lead enters the red blood cells, and little finds its way into urine, which is formed by the filtration of blood. Sweat and saliva, on the other hand, derive from the extracellular fluid pool — that is, the fluid that bathes the cells but is outside the circulatory system — so they have a higher lead content.

But we don't yet know enough to explain this finding satisfactorily. Dr Florence is therefore planning further work to look at how the body partitions lead that has been absorbed through the skin. He plans experiments using radioactively labelled lead in animals, and stable isotopes on himself and other volunteers. These distinctive forms of the element will enable him to follow the fate of the lead atoms after they have crossed the skin.

The importance of all of this is that workers in various industries, who are exposed to lead dust or fumes, may carry a significant load of inorganic lead on their skins. This may not be as safe as we had previously thought. If they are sweating — as well they may be in the conditions — then the element is likely to get into their bodies, even though it is in an inorganic form. Lead metal or inorganic compounds dissolve in sweat and then can react with lactic acid or various amino acids therein to form products that may be fat-soluble, so facilitating the passage of the lead across the skin.

## Metals in sweat

Sweat is the product of glands embedded throughout our skin and connected to the surface by small canals. The glands use the serum of blood as their starting point in producing sweat. Each one is supplied by a small network of blood capillaries.

The glands modify the serum — conserving glucose, but letting through various salts and small quantities of protein as well as waste products like lactic acid and urea.

Dr Florence and Dr Stauber conducted a series of experiments to measure the concentrations of four metals — zinc, cadmium, lead, and copper — in sweat. They found that, of the four, zinc had the highest concentration; next came copper, then lead, and finally cadmium. As might be expected, this order follows that of the metals in whole blood, determined previously by other scientists. However, in serum, the rankings differ slightly. This is because some of these trace metals, especially zinc, are found predominantly in the blood cells rather than in the fluid portion.

The absolute values for zinc, cadmium, and lead are very similar in sweat and serum, but copper is the odd one out. The reason is that, in serum, copper is tightly bound to a 'carrier protein' — ceruloplasmin — from which most of it does not easily separate to find its way into sweat.

In their study, Dr Florence and Dr Stauber compared the traces of the metals in sweat between males and females. They found that only the value for copper varied, whereas studies by other workers had shown differences for zinc and lead as well. Women had much less copper in their sweat than men did, but, interestingly, those women taking the contraceptive pill had almost exactly the same value as men.

Naturally, the scientists would like more data before they can be definite about the full extent of these differences or speculate on their causes.

But why bother to measure the minute amounts of these trace metals in sweat? Results may be interesting, but have they any application? The answer is a definite yes. Abnormal ratios of certain metals in serum can indicate physiological changes. For example, in leukaemia the ratio of copper to zinc changes. If we can be sure that the levels in sweat correlate in some way — not even directly — with those in serum, then sweat analysis would provide a convenient and 'non-invasive' way of diagnosing certain disorders.

Also, as discussed in the main article, a further application exists in the field of occupational health for workers in conditions where their environment contains certain metals in large amounts. Sweat analysis may provide a simple technique for checking levels of substances that, in excess, are very dangerous.

# Lead and your health

The proportion of ingested lead that actually remains in the body is very variable. The form of the element, the presence of other pollutants, and the general state of health can all influence it. The same applies to air-borne lead, although we do know that this contributes less to the body's total than ingested lead. However, contamination into the air, such as by lead in petrol, can result in lead-rich dust that settles on soil and crops and so enters the body in the diet.

Once absorbed, lead distributes itself around the body, but is not evenly stored. The skeleton bears the brunt, as lead only remains in the soft tissues for a few weeks, whereas in bone, with its slow metabolic rate, it can remain for as long as 30 years. Once there, it is relatively inert, but while in the soft tissues it can cause great damage.

Lead inhibits two enzymes that are involved in the synthesis of haem — the oxygen-carrying, iron-containing part of the pigment haemoglobin. Red cells therefore contain a reduced quantity of haemoglobin, and don't survive as long as usual. The result is anaemia, and an inadequate provision of oxygen to the tissues. In the blood, the enzymes start to be inhibited at lead concentrations of only 150 $\mu$g per litre, although significant anaemia does not occur until these reach 300 $\mu$g or more.

At even higher concentrations short-term lead poisoning also causes kidney and brain damage. Children are especially sensitive and, even if they survive it, an episode of poisoning can induce irreversible effects such as mental retardation and seizures. At lower levels, more subtle neurological problems — such as irritability and lack of co-ordination — occur.

Finally, at concentrations normally considered to be below those associated with known toxicity, some studies have suggested that lead can reduce intelligence in children. An example is a long-term experiment conducted by Dr Peter Baghurst of the CSIRO Division of Human Nutrition, together with researchers from the University of Adelaide, the Flinders Medical Centre, the Adelaide Children's Hospital, and the Child, Adolescent and Family Health Service. This followed 537 children born from 1979 to 1982 in and around the town of Port Pirie, situated downwind of a large lead smelter. Previous studies showed the topsoil and house dust in the district to be extensively contaminated with lead.

The team took blood samples from the mothers of these children during pregnancy, and from the babies at birth and at the ages of 6, 15, and 24 months, and every year after that, and measured the concentrations of lead therein. The scientists assessed the children's developmental status at the age of 4 years — in the areas of verbal ability, perceptual performance, memory and motor skills, and ability to judge quantities. They found that, on average, those children with more lead in their blood scored lower on these tests.

### What's in sweat

| compound | concentration (mg per litre) |
|---|---|
| sodium chloride | 800 |
| potassium chloride | 910 |
| calcium sulfate | 200 |
| magnesium sulfate | 88 |
| lactic acid | 1470 |
| urea | 300 |
| amino acids | |
| histidine | 240 |
| alanine | 920 |
| glycine | 280 |
| citrulline | 152 |
| protein | 350 |

Of course, in a study such as this (one of the most ambitious ever undertaken in the world), plenty of other factors come into play, but the researchers have tried to take these into account and have applied detailed statistical tests to be sure of the validity of the correlation that they observed.

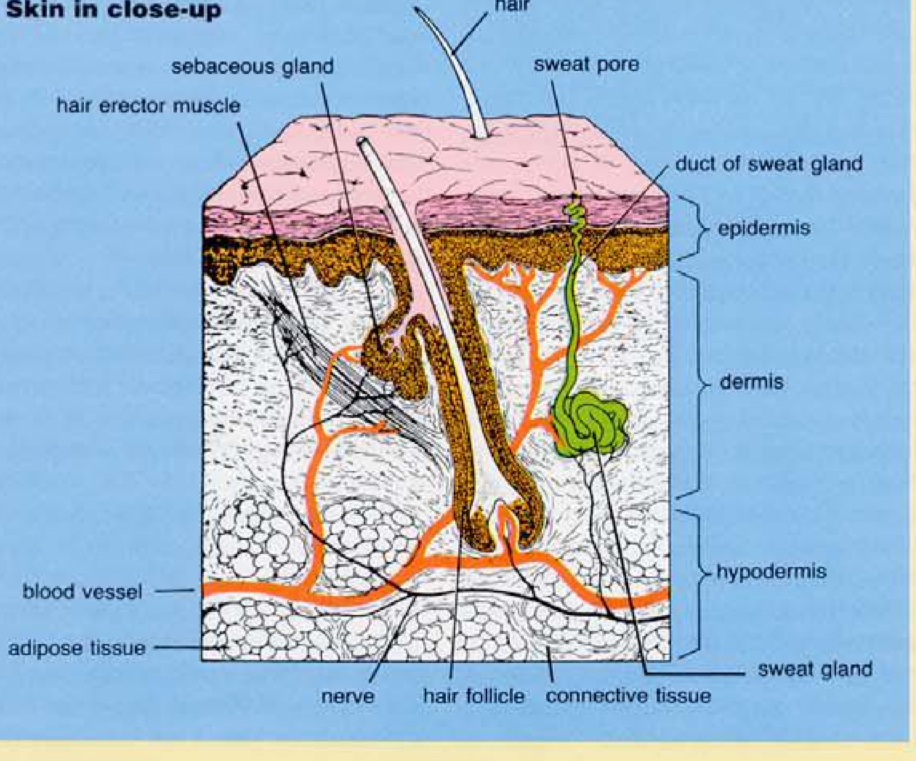
The work is continuing until all the children involved reach the age of 7, when they will take a final battery of tests. *Ecos* will keep you informed of the results.

The human body does not need lead at all, unlike some metals that, although toxic in large doses, are necessary for health in trace amounts. It seems the best policy is to avoid the stuff completely. We don't want to make the mistake of the ancient Romans, whose great civilisation used lead extensively, transporting their water in lead pipes and even flavouring their wine with it!

By studying Roman recipes and cooking utensils (lead-lined), scientists have calculated that a member of the aristocracy could ingest from 160 $\mu$g to 1·5 mg of lead per day. In this at least the plebeians were more fortunate than their masters, as they only consumed 35–320 $\mu$g.

Although some Romans started to realise the dangers of lead, it was very difficult to change the industrial habits of such a vast empire. And therein might have lain the seeds of their destruction. Knowing now the effects of the metal on the brain and the psychological state of some of Rome's rulers, it is not too far-fetched to wonder whether the demise of that civilisation was brought about partly by the actions of lead undermining the mental health of the aristocracy.

### Skin in close-up



**Volunteers sweat it out in the cause of science: sweat collection in a sauna (above) and (below) at a normal temperature, where sweating is induced in the forearm by iontophoresis.**

However, we don't yet know that lead taken into the body in this way is as toxic as when it is ingested. Because it leaves the blood-stream so quickly, and is excreted through sweat and saliva, it may not deposit in the body to the same extent. But even if such additions are not as toxic on a weight for weight basis, it obviously is not a good state of affairs to have any lead entering the body. The fact that lead metal or inorganic lead compounds can get across the skin is of considerable significance, and the conventional wisdom must clearly change.

*Roger Beckmann*

### More about the topic

The use of sweat to monitor lead absorption through the skin. S.G. Lilley, T.M. Florence, and J.L. Stauber. *The Science of the Total Environment*, 1988, **76**, 267–78.

The determination of trace metals in sweat by anodic stripping voltammetry. J.L. Stauber and T.M. Florence. *The Science of the Total Environment*, 1987, **60**, 263–71.

Skin absorption of lead. T.M. Florence, S.G. Lilley, and J.L. Stauber. *The Lancet*, 1988, **2** (8603), 157.