LEAD-POISONING IN CHILDREN

CORRELATION OF CLINICAL AND PATHOLOGICAL FINDINGS

BY

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Lead-poisoning in childhood is not a rare disease. The danger of ingestion of the metal exists from infancy, as shown by Clark (1950). Two fatal cases have been investigated at this hospital during the past year, and this paper is devoted to a description of the clinical and pathological findings. The changes in the kidney have received particular attention, as fatty vacuolation, which does not appear to have been a prominent feature in previously recorded cases, was found at both necropsies and an attempt has been made to correlate the biochemical findings in one case with the microscopical changes in the kidney.

The difficulty in making a diagnosis of lead-poisoning in infants is emphasized. Anderson (1948) points out that the central nervous system is particularly susceptible at this age, and states that after a relatively short period of ingestion of the metal the picture of encephalitis may develop in the absence of such well-recognized features as anaemia or colic. In both the cases described in this paper vague symptoms were present towards the end of the second year of life, and in neither was the diagnosis made at that time. In one case the child lived to the age of 4 years, when the presence of anaemia, constipation, abdominal colic, and punctate basophilia readily suggested the possibility of lead-poisoning, while in the other the diagnosis was made at necropsy.

The gross and microscopical pathological findings, notably the presence of intranuclear inclusions in the liver and kidneys, are described to assist in the rapid post-mortem diagnosis, and histochemical methods for detecting the presence of lead in the tissues are discussed.

Case 1

A white male child aged 4 years was first seen at the age of 2 years, when he appeared to be unsteady and tended to fall. No physical abnormality was detected at the time. He was again seen at the age of 4 years, when there was pain in the lower abdomen, pallor, and unsteadiness of six weeks' duration. Vomiting ensued and also frontal headache. The child was admitted to hospital and at this time was unconscious. The parents, when questioned, stated that, as long as they could remember, there had been a tendency to bite and chew the furniture and other articles, including toys.

Examination on admission showed a peculiar state of unconsciousness with widely staring eyes and continuous nystagmus. The skin had a grey colour, with a reddish tinge over the cheeks. There was excessive salivation, but the teeth were good and no lead line was detected. The tongue had a constant tremor, being protruded every few seconds, and there was twitching of the right cheek and the left side of the body. The blood pressure was 140/85 mm. Hg, and the pulse was rapid. Difficulty in seeing the fundi was experienced, but the veins were noted to be engorged.

The blood haemoglobin was found to be 60% (Haldane) and there was a leucocytosis with predominance of poly-

morphonuclear cells. Marked punctate basophilia was present. The urine contained a reducing substance which was identified as glucose by chromatography. A one-way chromatogram in phenol was made, taking the volume of protein-free urine corresponding to 250 μ g. of total nitrogen. After drying the strip and spraying with ninhydrin, excessive amino-aciduria was seen to be present. Two-way chromatograms were then run on Whatman No. 1 filter paper 18 by

Diagram of chromatogram of urine from Case 1. The sample has been placed at the bottom right-hand corner of the paper. Phenol has been run from right to left as first solvent and tetrahydrofurfural alcohol upwards as second solvent. The convention is used of representing the strength of the ninhydrin colour reaction by means of the size of the spot, using an arbitrary scale. 1, cysteic acid (from cystine); 2, serine; 3, glycine; 4, taurine; 5, alanine; 6, glutamine; 7, β -amino-*iso*-butyric acid; 8, leucine; 9, lysine.

22 in. (46 by 56 cm.), using phenol as first solvent followed by tetrahydrofurfural alcohol as second solvent. The result is shown in the diagram. The pattern shows an excess of cystine, taurine, and especially β -amino-*iso*-butyric acid. The identity of the latter two was confirmed by the copper method of Crumpler and Dent (1949). Cystine and β -amino-*iso*-butyric acid were the strongest spots on the square.

The other biochemical findings in this case were serum sodium 315 mg. per 100 ml.; potassium 16 mg. per 100 ml.; phosphorus, 5.8 mg. per 100 ml.; chlorides, 594 mg. NaCl per 100 ml.; bicarbonate, 41 vols. CO₂%. Blood urea, 60 mg. per 100 ml.; blood sugar, 0.127% glucose.

In the small quantity of urine obtained no porphyrins were detected.

X-ray examination of the long bones showed increased density of the metaphyses. Lumbar puncture yielded a clear fluid under increased pressure with a protein of 70 mg. per 100 ml., but no cells.

Death took place shortly after admission in spite of sedatives, surgical decompression, and attempts to lower the intracranial pressure by the use of hypertonic solutions.

Post-mortem Examination

Gross Findings.—The external features were not remarkable. There were ten teeth in the upper and lower jaws in good condition. The lungs were red, fleshy, and oedematous, the right weighing 163 g. (normal 90 g.), and the left 167 g. (normal 85 g.). The liver weighed 540 g. (normal 516 g.) and had a smooth yellow-brown capsule and a greasy yellow cut surface. The right kidney weighed 66 g. (normal 58 g.), and the left kidney 71 g. (normal 56 g.),

both organs having yellow-tinged cortical regions with poorly defined markings. Examination of the bones included the ribs, vertebrae, and lower end of the left femur. In the ribs and femur a dense white band several millimetres in width was seen in the diaphyses at the bone-cartilage junction. The brain weighed 1,330 g. (normal 1,190 g.), and was moderately engorged and tense. The white matter tended to swell on incision.

Microscopical Findings.—The principal changes were noted in the liver, kidney, and bones. In the liver the peripheral portions of the lobules were relatively intact, while the remainder showed extensive fatty vacuolation. Many of the hepatic cell nuclei were swollen and had a glassy appearance with wisps of chromatin below the nuclear membranes. Inclusions filling the greater part of the nuclei or several small inclusions were sometimes seen, and it was noted that the nuclear changes were most prominent in the neighbourhood of the portal regions, where there was less vacuolation. The smaller inclusions, in particular, showed variable acid-fast properties and stained orange with Masson's trichrome stain, as has been described by Wachstein (1949b), but for their detection it was felt that haematoxylin and eosin gave the best results. There was no cellular infiltration and the Kupffer cells were not mobilized.

The changes in the kidney were seen in the convoluted tubules, where the proximal portions appeared to be damaged, and in the medullary rays and outer portions of the medulla, where the looped tubules were thought to be involved. Fine vacuolation could readily be seen in the cytoplasm of the tubular cells, this feature being most marked near the basement membrane. Stains with Sudan black showed that the vacuoles contained fat. Some of the nuclei in the looped tubules were greatly enlarged and occasional inclusions were seen in this situation, but the latter were abundant in the proximal convoluted tubules.

The bone changes were studied in the rib and femur. The cartilage columns at the growing ends of the bones were short and regular. There was, however, inadequate erosion at the bone-cartilage junction, and broad, close-packed trabeculae were present for a distance of several millimetres along the shaft. This area corresponded with the white band noted in the gross specimen, and also with the zone of increased density noted in the x-ray films. There were numerous giant cells between the trabeculae, some of which had the appearance of typical osteoclasts, while others had swollen nuclei with clumped chromatin and a vacuolated or granular cytoplasm.

Demonstration of lead in the femur was found to be most satisfactory by the sulphide method described by Timm (1936). The tissues were fixed in absolute alcohol and decalcified in 30% formic acid. Excess acid was removed by 5% sodium sulphate and then washing in water. All the solutions were saturated with hydrogen sulphide. Frozen sections were cut and treated with potassium cyanide and yellow ammonium sulphide to remove copper, silver, and tin. The deposits of lead sulphide could readily be seen with the naked eye, and under the microscope fine black-brown granules were noted in the trabeculae. These granules were most abundant in the compact region close to the cartilage, but their presence could be detected for some centimetres along the shaft. Bismuth was excluded by staining with a 4% aqueous solution of potassium iodide and a 2% acidified aqueous solution of quinine sulphate as described by Lillie (1954).

Chemical analysis, using the method of Irving and Butler (1953), showed the presence of 158 mg. of lead per kg. of fresh bone in the femur. The total lead content of the liver was 9.205 mg.

The microscopical findings in the brain consisted of evidence of oedema with swollen glial cells in the white matter and lymphocytic infiltration in the meninges. A portion of the left popliteal nerve showed no unusual feature and there were no significant changes in the remaining organs, including the pancreas and parathyroids.

Case 2

A white male child aged 1 year 10 months gave a history of vomiting, anorexia, and constipation of one month's duration. He screamed and doubled up before vomiting, and was irritable, rubbing the back of his head and pulling at his ears. The mother stated that he appeared to be frightened, and constantly called out during the night. No history suggesting the possibility of lead ingestion was obtained. On admission to hospital he was very difficult to examine. He was ataxic and there was marked pallor. The fundi showed advanced papilloedema and engorgement of the retinal veins.

The blood haemoglobin was found to be 66% (Haldane) but there was no punctate basophilia. Sugar was present in the urine, and the spinal fluid contained 8 lymphocytes per c.mm. and had a protein content of 40 mg. per 100 ml. Ventricular taps were performed, but there was a great increase of intracranial pressure and convulsions developed. Death took place in spite of all measures, including operative decompression to relieve the intracranial tension.

Post-mortem Examination

Gross Findings.—The body was that of a well-nourished white male child. There were eight teeth in the upper and lower jaws with no evidence of a lead line on the gums. The right lung weighed 105 g. (normal 83 g.) and the left 93 g. (normal 74 g.), both organs being red, haemorrhagic, and fleshy. The liver weighed 411 g. (normal 345 g.) and was pink and yellow in colour with a rather greasy cut surface. The right kidney weighed 42 g. (normal 40 g.) and the left 45 g. (normal 43 g.), both organs being pink in colour with rather faint cortical markings. Examination of the bones, including the ribs, vertebral bodies, and the lower end of the left femur, was carried out. In the long bones a white thickened band several millimetres in width was noted at the growing end of the shaft. X-ray examination of the long bones *post mortem* showed zones of increased density. The brain weighed 1,360 g. (normal 1,050 g.) and was swollen with flattened gyri. The white matter was watery and tended to bulge above the cut surface.

Microscopical Findings.—The principal changes showed a similar distribution to those noted in Case 1. They were not so obvious in the liver, where the degree of fatty vacuolation was less and the intranuclear inclusions were smaller. The nuclear changes were, however, more widespread throughout the lobule, and the inclusions, though small, were more frequently seen. In the kidney the distribution of the lesions was again confined to the cortex and outer portion of the medulla, the proximal convoluted and looped tubules being involved. In the former the fatty vacuolation was finer and less prominent and there were no inclusions, while in the latter there were abundant inclusions and giant nuclei were readily noted.

The bone changes were similar to those seen in Case 1. The band of closely packed broad trabeculae was present with numerous typical and atypical osteoclasts. The lead was demonstrated in the lower end of the left femur by the sulphide method. The deposits of lead sulphide were much finer and less profuse than those in Case 1, and with the exception of the cortex they could be detected only for a distance of less than 1 cm. along the shaft. The changes in the brain were, by contrast, more marked. There was lymphocytic and large mononuclear cellular infiltration in the meninges. Large numbers of swollen glial cells were present in the white matter, and the latter showed evidence of oedema and softening, with focal accumulations of small glial cells and lymphocytes. The nerve cells in the brain stem showed fine cytoplasmic vacuolation and their nuclei had clumped chromatin with swollen eosinophilic but not acid-fast nucleoli.

No significant changes were found in the remaining organs, including the pancreas and parathyroid. The peripheral nerves were not examined. The lead content of bone (femur) was 101 mg. per kg. of fresh bone.

Discussion

The two cases described showed symptoms at the end of the second year of life. In the first case the patient was at that time restless and unsteady, and although his urine was not examined it seems probable that the cause of the symptoms was lead-poisoning. In both cases the clinical picture suggested cerebral damage, and it is of interest that the microscopical findings in the brain were more prominent in the younger child. Anderson (1948) emphasizes that the symptoms in young children may be confined to those indicating central nervous system involvement, and in the above cases ataxia, irritability, and restlessness at night would appear to be of importance. The symptoms, however, were vague, and it is felt that reliance must be placed upon the mother's statement that there is a change in the child's behaviour.

Punctate basophilia was present in only one of the two cases and, as shown by McKhann and Vogt (1933), too much reliance should not be placed on the presence or absence of this finding in arriving at a diagnosis. Abdominal pain was a feature in both cases, as was constipation, but in Case 1 these symptoms were not found when the child was first examined.

The gross necropsy findings were not striking in the cases described in this paper. There was some increase in weight in the liver and kidneys, this feature being attributed by some authors to the toxic effect of lead on mitosis (Chiodi and Cardeza, 1949; Finner and Calvery, 1939). This toxic effect would account for the giant nuclei shown in the renal tubules in the present cases. In order to arrive at a correct diagnosis in the necropsy room, a rib should be examined by dividing the bone longitudinally, when the white band close to the cartilage can be seen. For confirmation frozen sections of the liver and kidney may reveal the presence of intranuclear inclusions.

These inclusions were first described, in the livers of 21 children dying from lead-poisoning, by Blackman (1936), who showed that they could be produced in rats by the addition of lead to the diet. Similar inclusions have been found by Pappenheimer and Maechling (1934) in the cells of the renal tubules in a patient treated with bismuth, and Olitsky and Harford (1937) have shown that they occur after the injection of aluminium and ferric compounds. Wachstein (1949a) found that the metal-inducing inclusions were acid-fast, thus differing from other types of inclusions examined. This latter property was not a marked feature in the present cases, although this could be attributed to formalin fixation. Nuclear enlargement was, however, a prominent feature in the liver and kidney, some of the nuclei having a glassy appearance and others an apparently normal structure.

Previous reports of the kidney changes in lead-poisoning have described intranuclear inclusions in the tubular cells with nuclear hypertrophy. Wachstein (1949b) has found necrosis and calcification of the cells of the cortical tubules with foci of chronic inflammation in experimental work in rats. Fatty vacuolation does not appear to have been a feature.

In both of the present cases there was damage to the cortex and outer portion of the medulla involving the proximal convoluted and looped tubules. The findings consisted of fatty vacuolation of cytoplasm and swelling of nuclei with intranuclear inclusions. There was no evidence of cellular infiltration, and the collecting tubules and vessels were not remarkable.

The pattern of the urine chromatogram in Case 1 is similar to the one described previously by Wilson *et al.* (1953), especially as regards the large excess of β -amino-iso-butyric acid, which was the strongest spot on the square in this recent case. This amino-acid was originally discovered by Crumpler *et al.* (1951), who found that 5% of normal people excrete large amounts, and as the blood level of β -amino-iso-butyric acid in these people is no higher than in the rest of the population they assume that its excretion is probably due to a defective absorption in the renal tubules.

More recently, Sandler and Pare (1954) have made a study of this amino-acid and found that an increased excretion followed starvation. In a later paper (Pare and Sandler, 1954) these authors showed that cystine and β -amino-iso-butyric acid were excreted in excess in march haemoglobinuria and suggested that these two amino-acids together with haemoglobin are reabsorbed in the same or adjacent sections of the renal tubules.

Fanconi (1951) maintained that dextrose, phosphate, and amino-acids were absorbed in the proximal renal convoluted tubule. Wilson *et al.* (1953) showed that the amino-aciduria in lead-poisoning was renal in origin, as the level of plasma amino-acids was normal. The glycosuria and amino-aciduria which are a most frequent finding in cases of lead-poisoning have been concluded to be the result of injury to the proximal convoluted tubule. In both of the present cases the proximal tubules showed marked evidence of damage, thus confirming the work of Fanconi on the physiology of the kidney.

Summary

The clinical and post-mortem findings of two cases of lead-poisoning are reported.

The possibility of this disease must be borne in mind in all children with vague cerebral symptoms or evidence of increased intracranial tension.

Post mortem it is possible to make the diagnosis by examining the cut end of a rib or by examining frozen sections of liver and kidney for intranuclear inclusions and nuclear changes.

Fanconi's theory concerning the absorption of dextrose and amino-acids in the proximal renal tubules has been confirmed.

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The latest addition to the Medical Research Council's *Memorandum* series is called "Indices of Heat Stress" (No. 29, price 2s. net). Written by a statistician, it compares two indices used in measuring heat stress under hot and humid environmental conditions. The first, the "effective temperature," takes account of the temperature and humidity of the atmosphere, the rate of air movement, and radiation from the surroundings; the second is based on the rate of sweating. An earlier *Memorandum*, No. 17, describes the measurement of effective temperature. These memoranda will interest primarily those concerned with the well-being of workers in hot conditions. Both memoranda are published by Her Majesty's Stationery Office.