Urinary excretion of coproporphyrin in workers handling lead (*)

by

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The role of lead as an agent causing an occupational disease is mentioned by the Greeks, Romans Arabs since the remote times and its study up to the present has produced one the richest specialized medical literatures. Nevertheless, the majority of aspects of the pathological physiology of lead poisoning remain obscure or subject to considerable controversy.

The present paper is a contribution to the more adequate knowledge of the urinary porphyrin excretion in Brazilian workers handling lead. The quantitative value of this excretion was determined with the object of discovering the trades in which lead poisoning is more likely to occur.

It is now known that the urinary porphyrin observed in lead poisoning is coproporphyrin type III (1 to 6).

Garrod (1892) (7) and Stokvis (1895) (8) were the first to refer the increase in urinary porphyrins in human cases of lead poisoning. These findings were later confirmed by other workers.

METHOD

We used the method described by Schwartz, Hawkinson, Cohen and Watson (13), which is a modification of Fisher’s method, and is based on Saitlet’s observation that coproporphyrin is extracted by ether from urine acidified by acetic acid. The method is performed as follows:

1 — 100 c.c. of urine are acidified with 10 c.c. of glacial acetic acid and extracted successively 3 times with between one half and one volume of peroxide-free ethyl ether.
2 — The 3 fractions of are mixed and washed 3 times with 3% aqueous solution of sodium acetate.

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3 — Coproporphyrin is then extracted from the ether with 10 c.c. of 5% HCl, repeated 4 times.
4 — The acidity of the resulting solution is then reduced by the addition of a saturated solution of sodium acetate until it gives a slight red colour with Congo red paper.
5 — The coproporphyrin is then once more transferred to ethyl ether by 3 extractions.
6 — The ether fractions are mixed and washed twice with distilled water.
7 — The coproporphyrin is then extracted from the ether with 1N HCl, repeated 4 times, and making up a total volume of 25 c.c.

APPARATUS USED AND DETERMINATION OF THE CURVE

The total coproporphyrin was measured fluorometrically with a Lumetron photofluorometer model 402 EF. A violet filter was used for ultraviolet light, and two orange filters for fluorescence. As a diaphragm we used lower aperture 3 and higher aperture 6. As a standard for calibrating the No. 100 in the galvanometer we used a solution of coproporphyrin I (tetramethyl ester) sent by Professor G. Rimington to Dr. G. G. Vilela, to whom we express our thanks. A solution of sodium fluoresceinate with the same fluorescence as the solution of coproporphyrin was prepared for daily use. The results are shown in Figure 1.

NORMAL FIGURES

Table I gives the figures obtained by several workers who studied the urinary excretion of porphyrins, using, as a rule, the same technique, based on Fisher’s method.

To study the daily variation with the method used, we examined two normal men, one aged 24 and the other 34, testing for 10 successive days, recently obtained samples of 100 c.c. of urine obtained at the same hour of the day. The results, given in Table II, show that the variations are slight and that a single sample of 100 c.c. may be considered as representative.

PROBABLE ERROR WITH THE METHOD USED

Ten determinations were made with a single sample of urine from a workman (workman N.º 88) showing a high coproporphyrin level. The following results were obtained: (Table III).
Taking 90% of these results (sample No. 9 eliminated), an average of 8,966 gamma per thousand is obtained with an error of 5.2%, which is quite acceptable in fluorometric methods of determining an organic substance.

EXPERIMENTAL

In the present investigation, we examined workers engaged in 5 different trades, in all of which, exposure to lead was considered to be high: printing, production of pig lead for various uses (linotype, monotype, lead articles), manufacture of lead pipes, handling of lead paints, manipulation of tetra-ethylated petrol. All the workers examined were males, over 18 years old, as Brazilian law forbids the employment of women and men under this age in work where lead is handled. The samples of urine were always collected between 9 and 11 a. m., in the presence of one of the authors. Between the collection and the testing of the urines, never more than 3 hours elapsed.

BRIEF DESCRIPTION AND WORKING CONDITIONS OF THE ESTABLISHMENTS VISITED

Establishment A (printing) (Condition : bad)

Old building, two storeys, situated down town. It measures 9.20 m by 45 m. On the ground floor, which is cemented, are situated the office, the store, the printing machines and the stereotyping department. The upper storey has a wooden floor and houses the composing and binding departments, separated by wooden partitions 2 metres high. Tile roof over a wooden ceiling. There is natural ventilation on the upper storey through openings on the front and rear walls of the building. Front and rear walls: 3 windows opposite each other, 0.90 m wide and 2.10 m high. The rear windows lead to an area 1.50 m x 9.20 m. The ventilation of the ground floor is thorough the front doors and the back doors which lead to an are where toilet accommodation is situated, and is thus, inadequate. There is no artificial ventilation system. The stereotyping department is separated from the rest by a brick wall, and is situated at the front of the ground floor, occupying a small space of approximately 8 square metres. It is provided with 2 small casting ovens. The only opening leads to the street, measures 1.20 m x 1.50 m, and is closed by a glass window on a wooden frame, revolving on an horizontal axis.
ESTABLISHMENT B (printing) (Condition: very good)

Room 4.20 m wide by 7.10 m long, having in one of the shorter and one of the longer walls, wide openings from 1.20 m above the floor almost to the ceiling. The windows open on a park surrounding the building. They may be opened fully or the upper halves only, to avoid draughts blowing directly on the workers. When there is no wind, 3 oscillating fans hanging from the ceiling are in operation. Composing and printing are done in the centre of the room, and the linotypes and monotypes are at the sides. These machines are not provided with collectors.

ESTABLISHMENT C (Printing) (Condition: bad)

Large room, with wooden floor, on the first floor of an old building in the center of town. The room is rectangular, measuring 5.20 m x 9.80 m. Height 6.80 m. The left has 4 doors leading to a cemented corridor with 4 fixed glass windows. On the right side, which overlook the road, there are 3 openings 1.20 m x 2 m, spaced 1.50 m apart. In the center of the room, are situated the composing and printing departments and at the sides, the linotypes. In the left corner there is a small room in which there is a small oven for casting type, with a window opening on a courtyard. When the oven is working, fumes are produced, which penetrate into the main room, increasing the air pollution.

ESTABLISHMENT D (Stereotyping and monotyping) (Condition: bad)

Cemented room, measuring 6.20 m x 3 m, 2.80 m high, located behind the printing department, from which it is separated by a brick wall. In one corner there is a door 1.80 m wide. The door leading to the printing room is 2 m wide and is situated in the corner opposite to the door of the stereotyping room. The back wall has 3 openings, 0.80 m x 0.40 m, 1.80 m above the floor, 0.50 m apart from each other. Each window has 2 panes, hinged at their upper edges and opening outwards. In the room, towards the left, there is a casting ladle. There is no air conditioning system. To the right of the main room, is situated the monotyping department with 2 monotyping machine, in a room 2 m x 5 m.

ESTABLISHMENT E (Stereotyping) (Condition: very bad)

This was one of the worse places visited. It is situated at the right side of a printing room, from which it is separated by a wooden wall. It measures 2.20 m x 5 m. There are no openings in the walls. Casting is done
in a bay with a faulty exhaustion system which does not prevent the fumes from invading the room.

ESTABLISHMENT F (Tetra-ethylated petrol) (Condition: good)

Building situated by the sea, 3 m wide by 7 m long. Height 2.50 m. Roof of asbestos tiles, without ceiling. One of the longer and one of the shorter walls do not reach the roof. In the corresponding space above the two other walls, are fitted wire screens. The room is painted white and the standard of cleanliness is good.

ESTABLISHMENT G (Manufacture of lead pipes) (Condition: fair)

Big room measuring 18 m x 30 m, two thirds of which is used as a store for other departments of the factory. Height 6.80 m. Lean-to type of building, with 3 rows of clear glass roof lights spaced 5 cm apart. The side walls are not carried up to the roof, leaving a gap of 30 cm. There is a single lead melting ladle, with collector.

ESTABLISHMENT H (Manufacture of lead pipes) (Condition: very bad)

Cemented room 9 m x 5 m, connected to an open area, 9 sq. m. Two openings in the left wall. The upper floor is used as a store, and is 7 m wide, leaving thus a space 2 m wide between the iron railings at the outside edge of this floor and the right wall of the room.

ESTABLISHMENT I (Production of pig lead and lead pipes) (Condition: Lead pipes department: bad. Pig lead department: very bad)

Cemented room 25 m long by 8 m wide, connected with neighbouring buildings, and with an uncovered area at the center, 1.5 m wide. Entrance through an opening 2.20 m wide, starting from the left wall of the building. Height 6.80 m Roof covered with French tiles; no ceiling. Ventilating tiles above two furnaces. Three "windows", 1.20 m x 1.70 m, 1.50 m above the floor, in the front and back walls. The back hall overlooks a small stream. These "windows" are made of 2 cm wire netting in iron frames. The pig lead furnace (at the back) and the lead pipe furnace (at the front) are not provided with exhaustion systems.

ESTABLISHMENT J (Manufacture of lead pipes) (Condition: very bad).

Room 4.80 m wide by 20 m long, 4.50 m high. One entrance door only, 2 m wide. Tiled roof; no ceiling. No openings in the walls for ventilation. In the right corner at the back, there is an opening 1.50 m wide, leading to an area 2 m long and 4.80 m wide. One half of this area is roofed
with tiles and contain the toilets. The lead furnace is at the rear of the room, separated from the area by a wall without openings. The storing space is in the center of the room, the work shop and the office at the front. There is no artificial ventilation.

**ESTABLISHMENT K (Production of pig lead) (Condition: very bad)**

Situated at the center of an open space, the building is 5 m wide by 15 m long and has two entrances, one in the left wall, 2 m from the front, 2.20 m wide, and the other at the left corner of the back, 0.80 m wide. Height 5.20 m. Tiled roof. Two transparent glass windows the front, 1 m x 1.70 m hinged at the side. The front part is used as a store and at the back there are two lead furnaces, one small and the other of larger capacity. No artificial ventilation to improve the atmospheric physical conditions which are bad, due to the almost non-existent natural ventilatino.

**ESTABLISHMENT L (Manufacture of lead paints) (Condition: bad).**

Room 10m x 15m. Height 7.20m. The entrance, 2.40m. is in the center of the front wall, and is kept permanently open. In the same wall, there are 3 glass windows, 1.50m x 1.00m. opening horizontally. No openings in the other walls. The ovens are provided with waste recovery systems, and masks are given to the workmen engaged in inspecting, packing and cleaning.

**ESTABLISHMENT M (Manufacture of lead paints) (Condition: very bad).**

Square shed, 64m², roofed with tiles. Height 2.80m. The walls are 2m high. The entrance door is 2m wide. Built in the center of an open space, with good natural ventilation. The manufacturing methods are primitive; there is no exhaust equipment. The grinding of litharge is done in a stone mill placed at the center of the room. Minium is produced in an antiquated oven, without an efficient protection system, giving rise to considerable amount of dust. The workmen do not use the masks at their disposal.

**IMPRESSIONS OF THE HYGIENIC CONDITIONS**

The hygienic conditions of the establishments studied are, in general, defective, especially in the pig lead works (Establishments I, J and K) which may be considered as very bad. In these establishments, apart from faulty ventilation, the casting furnaces are not provided with any exhaust system to prevent the metallic fumes from heavily polluting the atmosphere, and the workmen have no masks. In the lead paint works (Establishments
L and M), although the workmen are provided with masks, they object to wearing them, thus increasing the contact with the dust produced during the various stages of manufacture.

The preparation of tetra-ethylated petrol was studied in only one concern (Establishment F) and it was concluded that the personal protection of the workmen is perfect. The good ventilation of the room and the use of one-piece white overalls, rubber gauntlets and masks, provides good protection for the workmen. On the other hand, the filling of drums with the product is not carried out under the same hygienic conditions, and we have seen, at times, the workmen engaged in this operation, not wearing the masks at their disposal.

RESULTS

The results obtained are shown in tables IV to XIV and in figure 2.

COMMENTS

The above data clearly show the influence of lead absorption on the excretion of coproporphyrin, which increases with atmospheric pollution by lead fumes and dusts, and is consequently closely related to hygienic conditions. This is clearly shown by the comparison between the establishments I, J and K — in which atmospheric pollution must be high, and where the excretion values are also high (average 6.088 micrograms per thousand ml) — and the establishments A, B and C, where both the air pollution and the excretion values are low (average 112 micrograms per thousand ml).

The study of workmen in establishments engaged in lead casting and manufacture of lead paints shows that this phenomenon is general involving all the workmen engaged in such unhealthy tasks. This fact leads to the inference that the excretion of coproporphyrin in persons who absorb lead, is general and constant, and consequently, that the determination of this excretion is a useful method not only as an indication of latent danger but also as a control of the efficacy of the measures used for the improvement of the working condition.

In the industries involving lead casting and manufacture of lead paints, all the workmen spoke of the dark colour of their urine, with only occasional clear samples. In cases N° 88 and N° 94 the urine was definitely “Port wine colour”.
The highest values of excretion, in cases of heavy exposure (production of pig lead and lead paints — see table XIII), reach the figure of 10,000 micrograms per thousand ml, which is only very occasionally exceeded.

An important result of the present study, is the evidence that the urinary excretion of coproporphyrin is an early symptom, as deduced from cases 83, 88, 93, 94, 95, 96, 99, 100, 104; 110; 113; 114 and 115; in which relatively soon after engagement high figures were observed. For a better study of this point, serial determination were done in urine samples of a workman, collected from the date of engagement. These findings were highly demonstrative and are presented in figure 2, in which are also shown the values obtained for other workmen from the same establishment employed for from 1 month to 20 years. The abnormal figure in the first sample (180 micrograms) may be explained by the former occupation of the workman (sheet metal work in bronze and other metals). These results confirm the observations of Carrié (17), Vannotti (25), Langen and ten Bergen (26), Salomon and Cowgill (27).

The printing trades, always considered as among the more dangerous, were not associated with great alterations on the excretion of porphyrins, and even these alterations were minimized by the good hygienic conditions in the works. In establishment B, for instance, in which sanitary conditions were very good, with excellent natural and artificial ventilation, all the figures were normal, both monotype workers (average 57 micrograms per thousand ml) and linotype workers (average 72 micrograms per thousand ml) show figures similar to printing press operators (average 60 micrograms per thousand ml). In establishment A, in which the conditions were inferior to the above, in 10 workmen we found 3 abnormal cases (cases 3, 4 and 7) (average 98 micrograms per thousand ml). As for establishment C, which is worse than establishment A (average 161 micrograms per thousand ml), and where the casting oven is in an adjoining room without any means of isolation, thus increasing the air pollution of the establishment, the excretion figures for the linotype workers are from 106 to 248 micrograms per thousand ml, and for the printers, from 106 to 298 micrograms per thousand ml.

In the case of linotype workers, differences were also found between the figures in establishments B average 57 micrograms per thousand ml) and D (average 361 micrograms per thousand ml), which can only be accounted for by a difference in hygienic conditions.
In contrast with these two trades, the stereotype workers always showed high figures, and these were associated with the hygienic conditions, as may be observed in the case of establishment E, the worst in this group.

In the manufacture of lead pipes the highest figures, observed in establishment H, seem to be due, not only to the faulty hygienic conditions, but also to the situation of the workmen in relation to the task performed. Whereas in the other two establishments (G and I), the pipes are collected by the workmen standing 1 metre below the opening of the furnace, in establishment G, the workmen are on a raised floor above the opening of the furnace, and thus more exposed to the fumes. The high figure observed for No 84 is due to the fact that he is in charge of inspection, and stands on a board used for charging the furnace, thus being in direct contact with the fumes produced.

The difference between the establishments L and M (manufacture of lead paints), is also clear. At the first, with an average of 4,200 gamma per thousand ml (even including the very high figure in case 110) the equipment is more modern, the high figures being due to deficient ventilation and the handling of the product without adequate protection. In establishment M a primitive method of production is used and the workmen invariably refuse to wear the masks with which they are provided; in this establishment, toilet facilities for the workmen are also deficient.

A trade that seems not to have been studied in this connection is the handling of tetraethylated petrol. Our results were obtained from workmen handling aviation petrol, the only petrol which is treated with tetraethyl lead in Brazil, as the ordinary petrol for motorcars arrives from the United States of America already treated. As already mentioned, the workmen employed in mixing the tetraethyl lead with petrol enjoy an excellent system of protection, which is subject to strict inspection. The distribution of the product into drums, however, is carried out under less strict inspection, and the workmen often do not use the means of protection at their disposal. It is our impression that the abnormal figures found (average 228 gamma per thousand ml) are due to this last factor.

A last point deserves special mention.

The investigator engaged in an inquiry into lead industries, expects from a reading of specialist books to find that workmen engaged for a certain number of years in this trade cannot help showing at least the majority, if not all the signs and symptoms described as indicating a state of lead poisoning. It is surprising to find workmen who for 10, 15, 20 and 30 years
have performed their duties normally, without complaints or showing only vague symptoms which may be due to a variety of causes. With the exception of establishment K, in which hygienic conditions were very bad, and where all the workmen complained of having suffered from abdominal pains at least once, the complaints were varied and usually vague, and the clinical signs were non-existent, even though in many cases the coproporphyrin level was above 1,000 micrograms per thousand ml.

In view of the vague clinical pictures found in workmen handling lead, the determination of coproporphyrin level should be of particular value, especially in cases involving legal disputes, in which, together with the other classical tests it may serve as a test of irrefutable evidence.

CONCLUSIONS

From the above, the following conclusions may be drawn:

1 — The typical clear picture of lead poisoning, as described in the classical text-books is rare in our experience.

2 — All the clinical signs and symptoms described as the result of the action of lead on the organism are not frequently observed on examination, which makes difficult clinical investigations on trades in which lead is handled.

3 — The increased urinary excretion of coproporphyrin, showing an intense metabolic disturbance, is the only physiological change resulting from the action of lead on the organism of workmen handling this metal.

4 — This increased coproporphyrin excretion is an early sign, and, as a rule, its appearance is independent of any other clinical sign or symptom indicative of lead absorption.

5 — The facility with which the test can be applied, and the correlation between coproporphyrin excretion and working conditions, make the determination of urinary coproporphyrin a safe tool in the study of the hygienic conditions of establishments in which lead is handled.