XXXIV.—*On Acetic and Formic Acid obtained from Human Urine during the Chemical Decomposition of Urochrome.*

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1. *Preliminary Notes.*—Proust was the first to obtain acetic acid, or as he termed it “vinegar,” by the decomposition of extract of urine. It was subsequently again obtained by Liebig from putrid urine, and believed by him to be a product of decomposition of the colouring matters. He did not obtain it from fresh urine, and believed that the other products of the decomposition of urochrome, the pitchy resins first described by Proust, could not be obtained from fresh urine, but were products of putrefaction. On p. 140 of the eleventh report of the Medical Officer of the Privy Council, 1869, I have enumerated the products of the chemical decomposition of urochrome, and amongst the volatile products I have enumerated acetic acid and uncertain volatile acids. Amongst the latter I have now recognized formic, and small quantities of higher acids. Formic acid had been repeatedly found to be an ingredient of human urine, but it had been declared an accidental product of the intentional ingestion into the stomach of certain substances which by decomposition in the economy yielded that acid. Moreover its presence had only been assumed on the basis of a few reactions

which did not exclude phenylic acid (which yielded similar reactions); it had not been found to be accompanied by acetic acid, which made the statement open to objection; and it had never been identified by its cardinal tests.

2. *Mode of obtaining Formic and Acetic Acid from Urine.*—These methods were first described by me in the Hastings prize essay, and may here be quoted:—

(a.) *From Fresh Urine.*—The urine is evaporated over the open fire to one-tenth, and filtered from the phosphates, urates, and gypsum. It is then slowly evaporated on the sand-bath, until a pellicle of urates forms. After cooling and filtering, it is evaporated on the water-bath to a syrup, and allowed to cool slowly over the water-bath, to effect a good crystallisation of chloride of sodium, urea, and other salts. The decanted syrup, mixed if necessary, with a little water, is treated with calcined magnesia, until alkaline, and until a filtered sample, treated with acetic acid and chloride of iron, does not give any immediate precipitate. The extract is then filtered. An extract thus prepared contains neither phosphoric nor uric acid, a diminished amount of sulphates, and a small amount only of chlorides. It contains free alkali and creatinine, but no magnesia. This extract of urine is put into a capacious beaker, and mixed with concentrated sulphuric acid, added, drop by drop, while the fluid is being agitated. A little uromelanine and some gypsum are precipitated in flocks. After filtration the fluid is diluted with water, and distilled in a capacious retort. When the fluid has been reduced to one-half, the black resin will be seen adhering to the sides of the glass, and to the platinum, which it is well to put into the retort to prevent bumping. The boiling is now interrupted, and the fluid allowed to cool. Fluid and resin are separated by decantation or filtration. The particles of resin are united by fusion in hot water. They are then washed and dried, and the resin is extracted from the black matter by solution in alcohol. The distillates containing the volatile acids, are treated as stated lower down.

(b.) *From Putrid Urine.*—Putrid urine is treated with a little lime in powder, or sawdust, and filtered. The dark brown filtrate is evaporated in an open dish over the open fire. Although it soon assumes a strongly acid reaction, nothing but ammonia passes away. All froth which rises during the evaporation is carefully skimmed off. When black particles begin

to appear on the surface it is allowed to cool, filtered, put into a retort, mixed with dilute sulphuric acid, and distilled. A mixture of hydrochloric, benzoic, acetic, and another acid passes over, together with a stinking matter, which deposits in flakes when the distillate is left to stand. The residue in the retort soon deposits the resin as a soft tar on the surface of the fluid, the sides of the vessel, and the platinum, put into the fluid for safe boiling. The resin is separated mechanically and by the filter, washed, and separated into its constituents by alcohol. The uropittine and uromelanine thus obtained present the same essential properties as those obtained from fresh urine.

3. *Treatment of the Distillates.*—Any solid benzoic acid (which occurs only in distillates from putrid urine, the distillates from fresh urine have all benzoic acid in solution) is filtered off. The fluid is then neutralized with powdered sodium carbonate and concentrated over the water-bath. When it becomes red and emits a powerful aromatic odour, it is allowed to cool and extracted with ether as long as this reagent extracts any of the essential volatile oil which yields the tyrosine reaction with mercuric nitrate on boiling. The solution of salts is then further concentrated to near crystallisation, and decomposed with an excess of sulphuric acid. The benzoic acid collects as a thick magma on the top of the fluid, and is filtered off. The liquid is mixed with water and distilled. The distillate is now already much purer, and is again neutralized, &c., and distilled once more. A pure solution of acids is now obtained which yields the reaction of both formic and acetic acid, but smells of butyric and caproic acid as well. Boiled with lead carbonate they yield lead salts, which sometimes crystallise, particularly with the help of a little ether, at other times remain as a colourless, transparent, thick and solid yet somewhat viscid mass. By no means, however varied, could pure crystals be obtained from the watery solution.

4. *Extraction of Neutral Lead Acetate by means of Alcohol.*—The watery solution of the first deposit from water was poured into alcohol. The mixture after standing some days deposited a large bulk of fine acicular crystals. They were isolated and allowed to drain on paper. When dry they contained (1.61 of moisture and) water of crystallisation, as follows:—

Analysis:—

(1.) 2.5725 grm. dried at 130°, fused, and frothed up, and

smelled faintly of vinegar. After many days' drying they lost 0.4052 grm. of water, leaving 2.1573 grm. of dry salt. Consequently the total loss was 15.81 per cent., and deducting from this the theoretical loss of $3\text{H}_2\text{O} = 14.2$ per cent., there remains a slight excess of moisture as stated.

(2.) 2.1573 grm. of the dry salt were treated with sulphuric acid, and then cautiously heated to redness, acid being once more added during an interval of the ignition. There remained 2.005 Pb SO₄, while theory would require 2.0102. The dry salt therefore contains 63.66 per cent. of lead, *i.e.*, the theoretical quantity required by neutral acetate.

5. *Extraction of Half-basic Lead Acetate from the Mixture of Lead Salts.*— $[2(\text{C}_4\text{H}_6\text{PbO}_4) + \text{PbO}]$ This salt has hitherto been obtained in chemical inquiries concerning acetic acid: (*a*) by heating the neutral salt to 280°, and keeping it at that temperature until the fused salt sets again; (*b*) by digesting the watery solution of two molecules of the neutral salt with one molecule of powdered lead oxide free from carbonic acid, until solution has taken place, and evaporating. The salt has an alkaline reaction. In the present research, however, this salt obtained as follows:—The acetic acid (containing formic) was treated with lead carbonate and boiled until saturated, and the filtrate was evaporated. At a certain point it crystallised entirely. After re-solution a small amount of salt crystallised first, and was separated and purified by recrystallisation. The saturated solution was now poured into a large amount of alcohol. Crystals deposited over night which were short prismatic needles of glassy lustre, gave on analysis the following results:—At 190° they lost a trifling amount of moisture, and remained constant; they were consequently anhydrous.

Analysis :—

(1.) .8698 grm. on combustion left .654 grm. of residue, being lead oxide = .434 grm. equal to .4028 Pb, and .22 metallic lead, total Pb = .6228 grm., or 71.60 per cent.

(2.) 1.1805 grm. left .8766 grm. residue, being .5456 PbO, equal to .5064 Pb, and .331 Pb, total Pb = .8374 or 70.93 per cent.

Mean of these analyses = 71.26 per cent. Pb.

Required by the above formula 71.37 per cent.

Schindler obtained a hydrated crystallised salt of this compound, which contained two atoms of water. He poured the

saturated watery solution into an equal or double amount of alcohol (strength not stated), when the salt was deposited in pearly scales. At 90° they lost 2 per cent. of water, fused, and on cooling formed a colourless gum. This when heated further was transformed into a white mass, losing more water. These forms of pearly scales, colourless gum, and white mass were also obtained by heating the solution of the salt from urine on the water-bath and letting it stand.

The circumstance that the crystals in the foregoing experiment were anhydrous, admits of explanation by the fact that a large volume of strong alcohol was employed in their separation. They moreover admit of no theory if considered to have contained any formiate.

But the *third* and *fourth* crystallisations were evident mixtures of neutral acetate and formiate; the latter crystallised in little opaque granular groups, while the acetate crystallised with brilliant crystals. The third crystallisation contained little, the fourth much formiate. The *fifth* crystallisation had a remarkably homogeneous appearance, and was analysed.

(1.) 1.1230 grm. yielded 1.1050 grm. $\text{PbSO}_4 = 67.22$ per cent. of lead.

(2.) 1.215 grm. yielded 1.9280 grm. $\text{PbSO}_4 = 67.67$ per cent. of lead.

Mean = 67.44 per cent. of lead.

In a mixture yielding the half-basic salt the quarter-basic could also be formed, which requires 76.95 per cent. of lead. But the fact that the third and fourth crystallisations contained increasing quantities of formiate made it probable that the fifth also contained formiate, although in appearance it was quite homogeneous. This was indeed established by a special examination, which yielded the reduction tests; and in one experiment with sulphuric acid so much carbonic acid and carbonic oxide was obtained, that the conclusion was unavoidable that the mixture of crystals contained at least one half by weight of formiate. In the sixth crystallisation, which also appeared quite uniform, the following relations were found—

Analysis:—

4.1580 grm yielded 4.1240 PbSO_4 equal to 67.74 per cent. Pb.

Consequently the lead had increased a little; the salt contained a little more formiate, which requires 69.6 per cent. of lead;

but even at the end no pure formiate could be obtained. The last mother-liquors contained small quantities of acid with less than the proportion of lead in the acetate. In short, in this instance the process of fractional crystallisation failed entirely to yield any pure product after the preponderance of acetate had ceased, and even the employment of alcohol did not effect that neat separation of formiate (insoluble) from acetate, (soluble in alcohol) which is advised in handbooks as if it were a fact; for the acetate was found to hold the formiate in solution even in alcohol, or to fall with it from the more concentrated solution.

6. *Barium Salts of the Volatile Acids from Urine.*—The lead salts having failed to afford the means of a neat separation of the acids, a new quantity of the acids was prepared and transformed into barium salts by boiling with barium carbonate. The solution was evaporated and when in a syrupy state was put aside for crystallisation. Crystalline crusts were slowly deposited, and removed. These first crystals were not analysed, but the next crystals (2nd crystals) were perfectly colourless and homogeneous and were analysed.

(1.) 3258 grm. gave 2992 grm. BaSO_4 , equal to 53.99 per cent. of barium.

(2.) 3998 grm. gave 0.3680 grm. BaSO_4 , equal to 54.12 per cent. of barium.

Theory for acetate, 53.72 per cent. of barium.

Mean found, 54.05 per cent. of barium.

This crystallisation, therefore, consists of pure acetate. It yielded all cardinal reactions of acetic acid with precision, and was free from formic.

The mother-liquor was now allowed to stand and formed a crust on its surface. Underneath that crust large beautiful crystals of rhombohedral shape, the obtuse corners cut off by a hemitropic (round principal axis) rhombohedron of greater elongation, were formed. They were of glass-like lustre and transparency. Some were detached entire and analysed separately.

Analysis:—

(1.) 7712 grm. lost at 110° 1010 grm. aq., or 13.09 per cent. From this it might have been supposed that the crystals were a new hydrate of barium acetate, of the formula $\text{C}_4\text{H}_6\text{BaO}_4 + \text{H}_2\text{O}$, At. W. 291, requiring 12.37 per cent. of water but—

(2.) The .3702 of dry salt yielded .6362 grm. BaSO_4 , equal to .3736 Ba. or 55.74 per cent. The theory of the acetate requires 53.72 per cent. The dihydrated barium acetate should by direct combustion, without previous drying, yield 47.07 per cent. Ba, but—

(3.) .7878 grm. (which frothed up on heating suddenly) left .66 BaSO_4 , equal to 49.25 per cent. Ba.

The crystals therefore contain, both in the anhydrous and dehydrated state, 2 per cent. Ba more than corresponds to acetate. The ordinary monohydrate of barium acetate, $\text{C}_4\text{H}_6\text{BaO}_4 + \text{H}_2\text{O}$, contains 6.59 per cent. aq., and 50.18 per cent. Ba. It was therefore probable that these crystals were not acetate, or not acetate only, and on examination it was found that they contained a considerable amount of formiate. The amount can be appreciated by putting the figures for Ba in acetate, crystals and formiate side by side—

Acetate requires	Crystals contain	Formiate requires
Ba 53.72 per cent.	Ba 55.74 per cent.	Ba 60.35 per cent.

The crystals correspond to a mixture of nine atoms of acetate with four of formiate, which requires 55.76 per cent. Ba. The form and stoichiometric relation of the water are probably an imitation of a new barium acetate dihydrate. *They show that barium acetate and barium formiate are isomorphous, and cannot be separated from each other by crystallisation in mixtures in which the atoms of formiate rise to more than one-third of the amount of atoms of the acetate.* The same was of course the case with the lead salts. Neither by distillation, nor by crystallisation of lead salts with and without alcohol, nor by crystallisation of barium salts, have I as yet obtained any pure formiate, although this salt is present in large quantity; but of the prevalent acetates pure crystallisations were obtained. I am therefore unable to determine directly the actual amount of formiates obtained as compared to acetates, but indirectly such determination may of course hereafter be made by destroying the formic acid, and distilling the remaining acetic. I estimate that upon five parts of acetic acid the urine yielded one part of formic.

7. *Determination of the Acetic and Formic Acid contained in the daily Urine of a Man.*—The total urine from eleven successive days of a healthy man was concentrated and distilled with sulphuric acid. A quantity of acid distillate was obtained, neutralized

with sodium carbonate and evaporated. The concentrated solution was again decomposed with sulphuric acid, and the benzoic acid separated. The liquid was again distilled, and the distillate boiled with lead carbonate. A quantity of salt was obtained which refused to crystallise, lost acetic acid during evaporation, and became at last partially insoluble in water. From a portion dibasic acetate crystallised. The whole was decomposed, and the amount of lead determined as sulphate. This weighed 8.007 grm., corresponding to 5.47 grm. of metallic lead. This corresponds to 8.59 grm. of lead acetate from urine of eleven days, equal to an excretion of 3.12 grm. in eleven days, or 0.288 grm. per day. I abstain from correcting the data for formic acid on account of what has been stated; its daily amount may be roughly estimated at .05 grm.

8. *On the Conditions in which Acetic and Formic Acid may be contained in Urine.*—As these acids can only be obtained from urine by processes and agents which effect evident decompositions, such as those of urochrome, it is fair to allow that the volatile acids also may, like benzoic acid,* be products of the cleavage of more complicated substances. But it is impossible at present to say what the substances are, and whether the acids are not simply present in combination with some base or other. The latter view might indeed be held if the relative significance could be attributed to the following reaction of urine, which I observed several years ago, and inquired into at some length.

Fresh urine is treated with some milk of lime and filtered; it is then treated with ferric chloride as long as a precipitate is thereby produced, and a slight excess of ferric chloride is added. The filtrate from the ferric hydrate will be found to have a deep red colour, similar to that which ferric salts produce in formiates, acetates, benzoates, succinates, sulphocyanides, and other salts. The reaction has lately been ascribed to sulphocyanide, but for this I sought years ago without success. I find now that it is yielded also by kryptophanates. Now the urine contains ammonia, which would form with ferric chloride

* *Benzoic acid* is most probably a normal ingredient of human urine. This excretion further contains a *peculiar benzoyl-compound*, differing widely from hippuric acid, which, under the influence of cold concentrated hydrochloric acid, quickly splits up into benzoic acid and the body which I have described as *omi choline*.

some basic salt, and this also might produce the reaction; the distillate from fresh urine, indeed, shows the reaction beautifully with a drop of ferric chloride. It is, therefore, quite impossible at present to say whether this reaction is due to any organic acid, much less to which of those which are now all (succinic acid is also alleged by Meissner to be constantly present in urine) shown to be contained in urine.

When urine or its extract is mixed with a moderate quantity of sulphuric acid and boiled, a very peculiar state of things ensues, which is not generally understood. The ingredients are not in the presence of sulphuric acid, as it were, at all, but in that of a very slight chemolytic influence only, a kind of contact action. The sulphuric acid is at once enveloped by the attraction of urea,* and as long as urea is present no sulphate is formed, except that of ammonia, even on boiling. Although some benzoyl-compound is decomposed in the extract of urine, and benzoic acid is given out, yet much hippuric acid remains in solution unchanged, and crystallises after the extract has been boiled for hours. If the hippuric acid had been in presence of free sulphuric or hydrochloric acid it must have been decomposed entirely by half an hour's boiling. To the same preserving influence of urea is due the fact that in the distillates obtained during the decomposition of urochrome according to my method, which yields the acetic and formic acid, *no hydrochloric acid is ever found*. Such acid is only evolved by the addition of a great excess of sulphuric acid to extract of urine, and its effect and advent are unmistakably evident in the fluid. *The mixture becomes black*, and loses entirely its previously red colour. It deposits black charred matter, which differs *toto calo* from uromelanine and the resinous urochrome products. From such charred urine no uromelanine and no pure products of any kind can be obtained. Now these particular conditions, more than anything else, incline me to the opinion that the acetic and formic acid are products of the decomposition of higher organic bodies, and not merely acids set free from salt by sulphuric acid. They escape simultaneously with the carbonic acid gas which is

* Presuming the urine to contain urea, which does not appear to be proved. If the urea, isolated by various processes, were the result of the splitting up of a more complicated body, then the neutralizing influence upon the sulphuric acid here alluded to would of course have to be exercised by this compound of high atomic weight.

evolved from the urea, and causes a continuous slight effervescence in the fluid. The sulphuric acid does not decompose the kryptophanic acid in the extract any more than the hippuric, *i.e.*, only a portion of it, and that certainly a small one. But when free hydrochloric acid appears the kryptophanic acid disappears, forming, in fact, the great bulk of the black matters in which such decomposing extract of urine abounds. These peculiar reactions must be carefully borne in mind by every one who would successfully study or comprehend these decompositions.

The foregoing research has been conducted by me for the Medical Department of the Privy Council, at the Pathological Laboratory of St. Thomas's Hospital.