

## A RAPID METHOD FOR SEPARATING HIPPURIC ACID FROM URINE

By HERBERT E. ROAF, M.D., *Lecturer on Physiology.*

*From the Physiological Department, University of Liverpool*

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When hippuric acid is precipitated by acid from a dilute solution of one of its salts it usually separates slowly, and when obtained from urine it also carries down with it a certain amount of pigment.<sup>1</sup>

The following method has been found a convenient way in which to prepare hippuric acid from (herbivorous) urine. It consists, in short, in adding ammonium sulphate to the urine before acidification. By this means the hippuric acid crystallises out rapidly, and usually contains only a comparatively small amount of adherent pigment.<sup>2</sup> This can be removed, and the crystals obtained free from pigment, by recrystallisation, after boiling the free acid (or its sodium salt) with animal charcoal.

The following experiments will illustrate the results which can be obtained by altering the conditions of experimentation. To samples of twenty-five cubic centimetres of cow's urine varying amounts of salt and acid were added, and the time at which crystallisation commenced was noted. In some cases the crystals were filtered off after a definite period and the filtrate allowed to stand for twenty-four hours to see if a further crop resulted.

1. For the usual methods of preparing hippuric acid see *Naubauer u. Vogel*, *Harn Analyse*, 10th ed. p. 225, 1899.

2. Previous treatment by heating with milk of lime and then filtering did not appear to lessen the amount of pigment in the crystals.

	Urine	Salt	Acid	Crystallisation commenced	Remarks
1	25 c.c.	—	1 c.c. 31 % HCl	—	} Only one or two deeply pigmented crystals at the end of 24 hours
2	25 c.c.	—	1 c.c. 31 % H <sub>2</sub> SO <sub>4</sub>	—	
3	25 c.c.	6 g. NH <sub>4</sub> Cl	1 c.c. 31 % H <sub>2</sub> SO <sub>4</sub>	—	No crystals recognisable at end of 24 hours
4	25 c.c.	6 g. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1 c.c. 31 % H <sub>2</sub> SO <sub>4</sub>	15 mins.	Good yield in 35 mins., not complete in 1 hour
5	25 c.c.	6 g. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	2 c.c. 31 % H <sub>2</sub> SO <sub>4</sub>	20 mins.	Tends to become pigmented, good yield
6	25 c.c.	6 g. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1 c.c. 98 % H <sub>2</sub> SO <sub>4</sub>	20 mins.	Slowly becomes pigmented, good yield
7	25 c.c.	8 g. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1 c.c. 31 % H <sub>2</sub> SO <sub>4</sub>	10 mins.	Good yield in 30 mins., not complete in 1 hour
8	25 c.c.	6 g. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1 c.c. 31 % HCl	15-20 mins.	Good yield in 30 mins., not complete in 1 hour
9	25 c.c.	6 g. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.75 c.c. 31 % HCl	10 mins.	Not so good a yield as in No. 8
10	25 c.c.	6 g. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	2 c.c. 31 % HCl	15 mins.	Crystallisation slow and incomplete, crystals pigmented
11	25 c.c.	8 g. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1 c.c. 31 % HCl	10 mins.	Good yield
12	25 c.c.	12 g. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1 c.c. 31 % HCl	5 mins.	Crystallisation complete in 10 mins.

From this table one sees that the addition of ammonium sulphate facilitates crystallisation, whilst ammonium chloride has no such effect. The greater the amount of ammonium sulphate the more rapid the crystallisation, and when saturation is approached the crystals are completely formed in ten minutes, so that on filtering them off no further crystallisation occurs, in the filtrate, even if it is allowed to stand for twenty-four hours. The amount and nature of the acid used has an influence on the rapidity of crystallisation. Using sulphuric acid it was found that increase of acid had practically no effect on the rate at which the crystals formed, but with hydrochloric acid it was found that increase of acid retarded the onset of crystallisation. Thus, the only disadvantage of adding excess of sulphuric acid is to cause an increase of pigmentation, whilst an excess of hydrochloric acid has the additional drawback of delaying crystallisation. It is, however, necessary to add sufficient excess of acid to completely separate hippuric acid from its salts (see experiments Nos. 8 and 9).

The delay of crystallisation caused by too large a quantity of hydrochloric acid was at first thought to be due to the hydrogen ion, but that idea is negated by the experiments using larger quantities of sulphuric acid (Nos. 5 and 6). The true explanation

probably lies in some action of the chlorine ion, because a more marked delay is shown when ammonium chloride is substituted for ammonium sulphate (No. 3). This action is rather curious as it presents an analogy to the action of ammonium sulphate in precipitating proteins more readily than does ammonium chloride.

With large amounts of ammonium sulphate and not too large an amount of acid it naturally makes no difference whether hydrochloric or sulphuric acid is used. It is, however, safer to use sulphuric acid throughout the procedure so as to avoid any danger of delay by adding too large an amount of hydrochloric acid.

The best proportions for most purposes are 250 grammes ammonium sulphate (or an equal volume of saturated solution) and 15 c.c. concentrated (98 per cent.) sulphuric acid to each litre of urine. This mixture is allowed to stand for twenty-four hours and the crystals filtered off. When more rapid crystallisation is desired the amount of ammonium sulphate can be increased. The method has been found applicable to prepare hippuric acid from large volumes of urine and also, as a class experiment, to separate it and obtain its characteristic reactions using so small a quantity as 25 c.c. of urine.