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Carrell R W & Kay R. A simple method for the detection of unstable haemoglobins.

*Brit. J. Haematol.* 23:615-19, 1972.

[Biochemistry Unit, Pathology Department, Christchurch Hospital, New Zealand]

Many abnormal haemoglobins that cause disease have decreased molecular stability. This paper describes the detection and isolation of these unstable haemoglobins by their preferential precipitation from blood lysates incubated at 37° C in a 17 percent v/v isopropanol solution. [The *SCI*® indicates that this paper has been cited in over 300 publications.]

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In 1968 I returned to New Zealand after a period in Cambridge, England, where I had carried out work with Hermann Lehmann that led to the description of a new disease syndrome, the unstable-haemoglobin anaemias.<sup>1</sup> From this work, it became clear that many of the genetic variants of human haemoglobin that cause disease do not show significant electrophoretic abnormalities. However, most do have conformational changes that affect their molecular stability. The traditional method of detecting this change in stability was by incubation of the lysed blood at 55° C. In practice, it was difficult to persuade technicians to regularly use the 55° test, the reason being that the need for the test often arises late in the day, too late to heat and stabilize a water bath at 55° C. Because of this problem, I set out to devise a test of haemoglobin stability that would utilise a 37° C water bath, which is always available even in the most basic clinical laboratory.

The method chosen was based on the premise that the lesions perturbing conformational stability were primarily those affecting steric/hydrophobic bonding and,

consequently, small changes in the polarity of the surrounding solvent should result in unfolding and denaturation of the abnormal haemoglobin. After some pilot experiments with isopropanol-aqueous mixtures, the problem was handed to an undergraduate student, Robert Kay, as a two-month project. He showed that a solution of 17 volumes of isopropanol made up to 100 volumes with aqueous buffer gave unequivocal precipitation of unstable haemoglobins after less than 30 minutes of incubation at 37° C. The test has subsequently been widely used and quoted, particularly in clinical laboratories,<sup>2</sup> and has resulted in the detection and isolation of numerous variants of haemoglobin that cause disease.

A subsequent adjunct to the isopropanol test<sup>3</sup> is the commercial availability of standards formed by the complexing of normal haemoglobin with zinc. These standards provide confidence for the inexperienced technician in the interpretation of the test.

There has been some rewarding feedback from the paper. It was flattering to learn that in many countries, including, to my surprise, Mongolia, the procedure is known as the Carrell test. I should warn others, though, that there is also an amusing side to the attachment of your name to a test, since it seems to be followed before too long by an expectation that you are either dead or nigh unto that state!

The most pleasing anecdote about the test was recounted to me by a young French doctor. Soon after the procedure had been published, he commenced studies on abnormal haemoglobins in France, which were interrupted when he received his call-up for military service in North Africa. His professor suggested that he continue his electrophoretic screening there, but to his dismay, he found he was posted to an area without electricity. With typical Gallic flair, he converted the survey to the isopropanol test and was rewarded by the finding, in the first batch of samples, of a new unstable haemoglobin.

Kay went on to complete his biochemical training and is now a genetic engineer in Boston. For me, the paper was helpful in establishing my work in molecular pathology in New Zealand and this, in turn, led to my recent return to the University of Cambridge as professor of haematology.

1. Carrell R W & Lehmann H. The unstable haemoglobin haemolytic anaemias. *Semin. Hematol.* 6:116-32, 1969. (Cited 85 times.)

2. Zinkham W H. Unstable hemoglobins and the selective hemolytic action of sulfonamides. (Editorial.) *Arch. Intern. Med.* 137:1365-6, 1977.

3. Carrell R W. Methods of determining haemoglobin instability. The unstable haemoglobins. (Huisman T H J, ed.) *Methods in hematology*. Edinburgh: Churchill Livingstone, 1986. Vol. 11. (In press.)