S2-1 03, 1972: Criteria of purity

Clip: Jeff Haywood demonstrates paper chromatography and high voltage electrophoresis

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Jeff Haywood:

I have to wear gloves for this process. Otherwise, the proteins on my hands will come off onto the paper and would probably spoil the results. In fact, I put my hand on a piece of paper and stained it for protein, and that's what I got – a real hand-print.

Well, we take our spot of hydrolised protein fragments and put them in one corner of the piece of paper. Then we want to separate the fragments up the paper, and so we'll dip it into the bottom of a trough containing a suitable liquid which will soak up the paper and carry with it the fragments. It carries them at different speeds, and so we can effect a separation.

Now, because we want to perform this on three protein samples – and if possible we want to treat them identically – we'll do it in this kind of an assembly. It just holds the papers vertically, and separate from each other, and it allows us to immerse it to a known depth. We'll put it in a tank, mostly because the solvents will evaporate away from the surface of the paper, giving us an uneven effect, and there will also not be a constant vapour pressure between the surface of the liquid and the air. So we'll seal it with a lid.

There's one further precaution. The fumes are harmful to me, so we'll do it inside a fume cupboard to keep it safe.

Well, that'll take a few hours to run. And if we run it and then stain it, we'll get something which looks a bit like this. We've got all the fragments spread up this line of the paper, but they're not very discrete… they're still rather close together, and there could be more than one fragment there in any one spot. So we want to spread them out more. Well, we could turn the paper over, put it back into chromatography and run it back up, but it wouldn't gain us very much. We'd be better to use a different criterion for separation, and that's what we'll do. We'll use the criterion of separation 'charge', and we'll use high voltage electrophoresis to do it.

This is the machine that we do the high voltage electrophoresis in. We have a high voltage stabilised power pack capable of generating about 5,000 volts, and an assembly here into which the paper goes.

At each side, we have a trough containing buffer, as we did with the gel electrophoresis, and the electrode is in it. In the centre, we have a block which is cooled by circulation, because the heat generated during the process is quite large. Now, because paper is a good insulator, we're going to have to soak it to allow the current to pass through.

So I take my soaked paper, which has already been run by chromatography in one direction, apply it in the opposite direction… I'll make contact with the 'wicks'… and close the lid. I can't actually switch the power on until this lid's down, because there's a safety switch involved. It'll take a while to run, you can't run this sort of thing in a short time. So I did some earlier, I sprayed them, and I hung them up in the oven to develop.

And here they are. Developed fingerprints. That's the sickle-cell haemoglobin… and that's the normal adult haemoglobin.