COAXING RUBBER TREE PROTEINS OUT OF BACTERIA

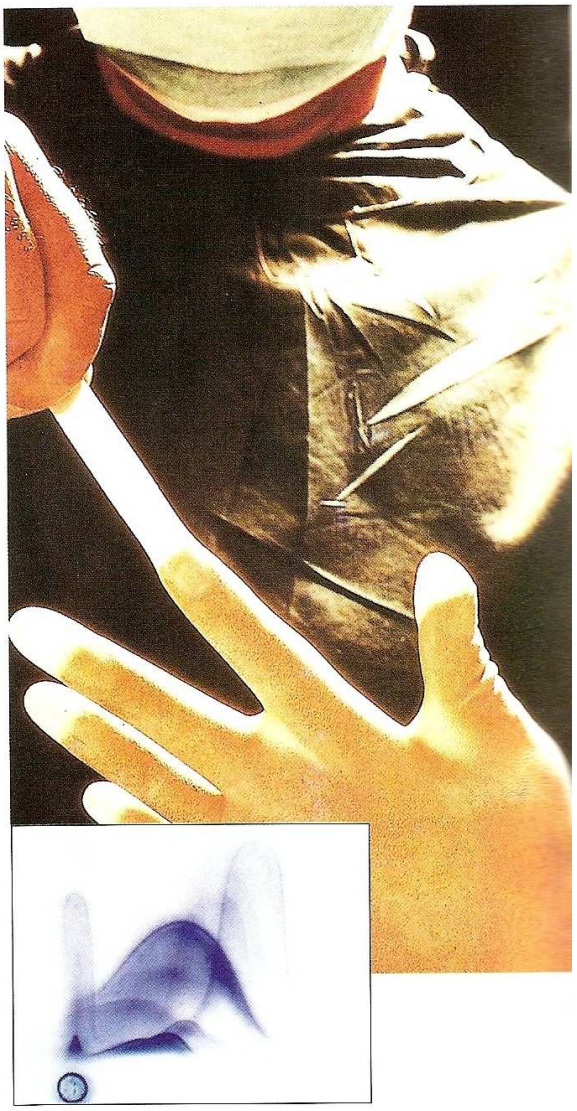
In 1993, the problem of latex allergy was being viewed with rising concern among healthcare workers who used latex gloves continually. Glove manufacturers in Malaysia were no less anxious about how the problem might affect the RM2.5 billion (about US$1 billion) latex industry. In the early days of latex allergy research, it was already recognised that the allergic reaction was triggered by specific latex proteins. But which one? As over a hundred proteins were found in natural rubber latex, the specific proteins(s) responsible was still unknown. The task of identifying the allergen (the protein that caused allergy) was more than simply another academic exercise. Once the allergen was pinpointed, it would be possible to develop diagnostics for latex allergy and to develop immunological assays to assess the level of the allergen in manufactured latex goods. Researchers and physicians took notice, therefore, when a group of scientists in Germany reported that they had identified the specific protein responsible for latex allergy.

According to the report, the allergenic protein in *Hevea* latex was the 'Rubber Elongation Factor' (REF), a previously known protein that was located on the membrane of rubber particles. Barely had this result been published when another group comprising Canadian and American researchers raised doubts on the finding. While further study was expected to resolve this discordance, a potential problem was anticipated in the preparation of highly purified REF needed for the tests. Natural rubber latex contains a complex mixture of proteins and so the likelihood that other latex proteins co-purifying with the REF could not be overlooked. Since even small amounts of protein allergens could trigger an allergenic reaction, these interloping proteins, rather than REF, might turn out to be the real culprits. It was at this juncture that RRIM researchers decided to have a hand at resolving the controversy. They realised that the only certain way REF could be prepared absolutely free of contaminating latex proteins was *not* to prepare it from natural rubber latex.

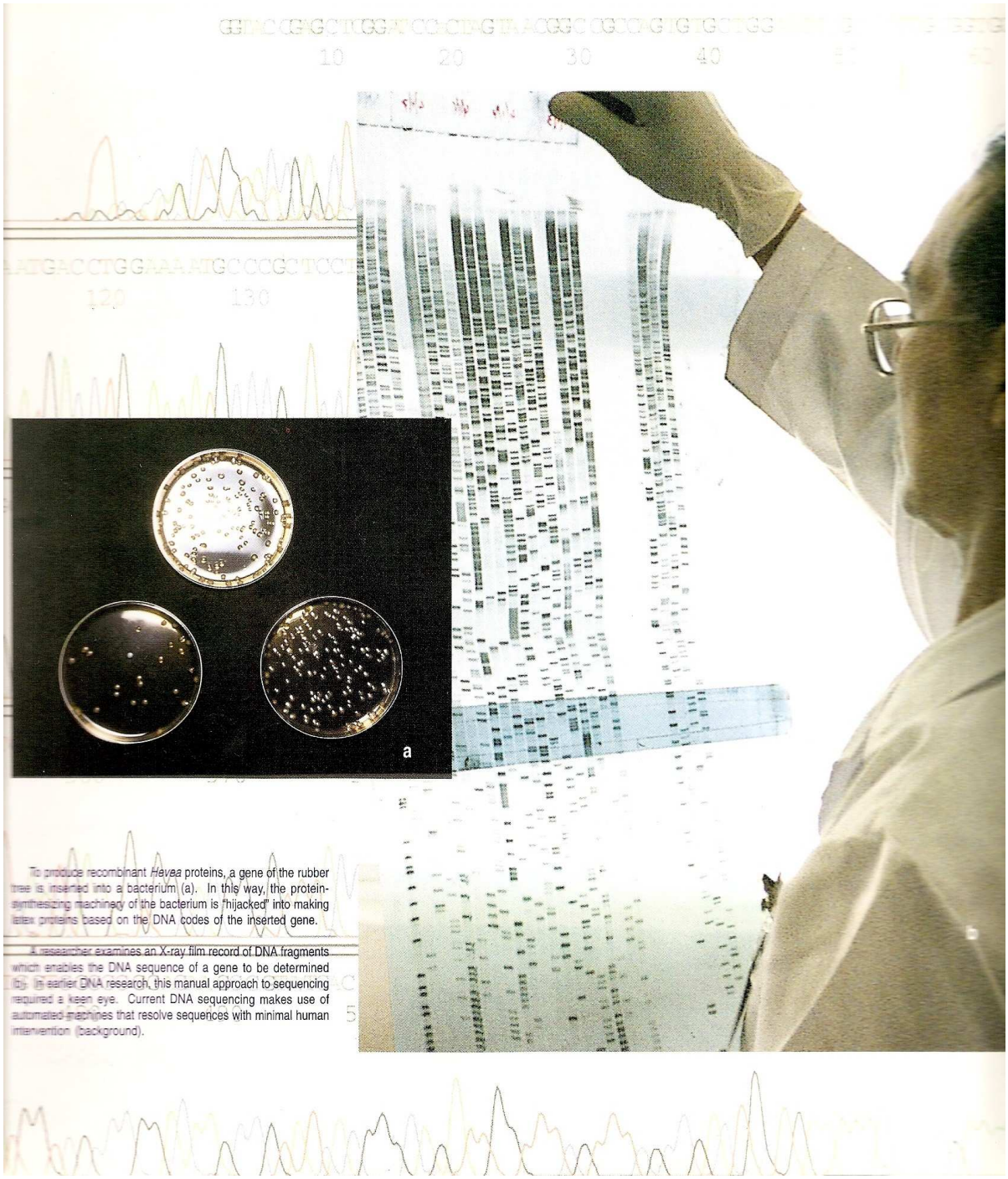
RRIM researchers did in fact make use of latex as the starting material for their experiments, but what they isolated from the latex was not the REF protein, but the specific DNA that encoded REF. This DNA was subsequently spliced on to bacterial DNA that would provide instruction for protein synthesis. The resulting DNA composite was then inserted into bacteria that acted as surrogates for the rubber tree to produce a recombinant ('synthetic') REF protein. In essence, what RRIM researchers did was to harness the protein-synthesising machinery of the bacterium to manufacture a rubber tree latex protein following the REF's DNA blueprints that had been inserted into the microbe. The recombinant protein so produced was assuredly free of contamination from other latex proteins because it did not come from latex in the first place.

The results of the RRIM's tests using recombinant REF showed that the two differing groups were partially correct. REF was indeed an allergenic protein, but it was not the major latex allergen originally depicted. Patients with *spina bifida* were found to be particularly allergic to REF, whereas healthcare workers - by far the larger group of latex-allergic patients - were considerably less commonly sensitised to the protein.

Subsequent to this work, ten latex allergens have been identified, including three by the RRIM working in collaboration with Universiti Sains Malaysia. While several recombinant latex allergens have since been successfully synthesised in laboratories worldwide, the REF was the first functional recombinant latex allergen produced. This work also marked the first occasion that RRIM employed recombinant DNA technology to answer a research question of the rubber industry.



A small number of people, mainly healthcare workers in the West who don latex gloves habitually, become allergic to latex. This has prompted research into latex allergy at the RRIM. Inset: A mixture of proteins eluted from latex gloves are separated by two-dimensional immuno-electrophoresis. Individual proteins that can induce an immunological reaction appear as peaks.



To produce recombinant *Hevea* proteins, a gene of the rubber tree is inserted into a bacterium (a). In this way, the protein-synthesizing machinery of the bacterium is "hijacked" into making latex proteins based on the DNA codes of the inserted gene.

A researcher examines an X-ray film record of DNA fragments which enables the DNA sequence of a gene to be determined (b). In earlier DNA research, this manual approach to sequencing required a keen eye. Current DNA sequencing makes use of automated machines that resolve sequences with minimal human intervention (background).