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PERSONAL EXPERIENCES DURING DISCOVERIES IN SCHISTOSOMIASIS

JR KUSEL

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Title:

Personal experiences during discoveries in Schistosomiasis

Authors:

JR Kusel[1]*, L Vieira[2], W Harnett[3], A Ruppel[4], D Cioli[5], M Doenhoff[6], D Colley[7], PMZ Coelho[8], RA Wilson[9] and EG Cox[10]

Affliations:

[1]Division of Infection and Immunity, Medical and Veterinary Life Sciences, University of Glasgow, Biomedical Research Centre, 120 University Place, Glasgow, G12 8TA, UK

[2]Department of Biochemistry and Immunology, Institute of Biological Sciences, Block O4 room 189 UFMG 31270-901, Belo Horizonte MG, Brazil

[3]Professor of Molecular Immunology, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, UK

[4]Institute of Public Health, University of Heidelberg, D-69120 Heidelberg, Germany

[5]Institute of Cell Biology, CNR 32 via Ramarini, 00015 Monterotondo (ROMA), Italy

[6]Senior Research and Teaching Fellow, School of Life Sciences, Room B110, School of Life Sciences, Building University of Nottingham University Park Nottingham NG7 2RD Dan Colley, UK

[7]Professor and Director, Department of Microbiology, University of Georgia, Center for Tropical and Emerging Grobal Diseases, 145 Coverdell Center (706) 542-4112, Athens, GA, USA

[8]René Rachou Research Center, CPqRR - Fiocruz in Minas Gerais, Rene Rachou Research Center, CPqRR - The Oswaldo Cruz Foundation in the State of Minas Gerais, Brazil

[9]Emeritus Professor, Department of Biology, University of York, Heslington York, YO10 5DD, UK

[10]LSHTM, Keppel Street, London WC1E7, UK

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***Corresponding author:** John Robert Kusel, Professor, Division of Infection and Immunity, Medical and Veterinary Life Sciences, University of Glasgow, Biomedical Research Centre, 120 University Place, Glasgow, G12 8TA, UK, Tel: 01236727349; Mobile: 07731898431; E-mail: John.kusel@glasgow.ac.uk

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1 SUMMARY

The life and work of Leiper and Leishman was celebrated in a Symposium in January 2016 (Glasgow Encounters with Tropical diseases). This symposium has stimulated us to record our personal experiences in our research in schistosomiasis and other parasites which for all scientists herein has occupied a lifetime. Our aim is to show the way research flourishes or flounders and the different emotions which discoveries can generate. The role of serendipity is well represented. The scientific themes are described in some detail and include the host antigen hypothesis, the surface membrane, the hygiene hypothesis, the immunology of human infections, heterologous and concomitant immunity, snail control and immunity, mechanisms of drug action, the excretory system, the oesophagus and feeding of the adult worm, immuno-diagnosis and vaccine production. We conclude that the awe and wonder about the living parasite and its interaction with the host is a major element in the experiences of the scientists involved. Students and close colleagues also play essential roles.

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2 INTRODUCTION

The discoveries of Leiper, celebrated in the Glasgow Symposium in January 2016, have been carefully and imaginatively described by [1]. The account by [2], has also gripped our imagination. Leiper's success was helped by his doggedness and thorough methods and also relied on the help he was given by Miyairi and Suzuki, the Japanese scientists he met in 1914. All this has stimulated us to assemble some personal accounts of discoveries in the field of schistosomiasis and other parasitic diseases. Each scientific worker has been asked to give an account of their work in the form of a story, as an adventure, in which the thoughts of the scientist at the time of the research are made clear. This is so that we can understand the history of the discovery, the effects that the work has on the scientist, and in some cases the visions which flow from the activity. We wish to portray research as a dynamic and very personal affair. Personal emotions during scientific research are not often recorded in historical accounts but it is clear from the stories given here and that of [2], that scientists are very caught up in emotions during and after discovery. In this article examples are drawn from scientists who have made significant discoveries in schistosomiasis and other parasitic diseases.

The discovery

All scientists have made an observation that illuminates their lives. This revelation concerns the problem they are tackling and is both an intellectual and an emotional experience. It may come after a great deal of worry and the search for an answer can be incubated for a very long time. From this observation a whole world of ideas and experiments suggest themselves and I form a hypothesis.

Testing the hypothesis

This is the essence of the scientific approach and when I do this I am conscious of a highly exciting intellectual activity. I am also conscious that I may be attaching some emotion to my commitment to this hypothesis. I may feel "certain" that I am on the right track-this can mislead the scientist and often scientists are unwilling to renounce their own ideas.

In this activity, an "Act of faith" is necessary for the immense intellectual and physical effort required to complete the necessary work. The formulation of the hypothesis often has involved a creative intuition and the choice of subject may involve a great feeling of compassion and emotion. This idea has been well described and discussed in Michael Polanyi's "Personal Knowledge" (especially under "Intellectual Passions" Polanyi) So the scientist is certainly using spiritual qualities, committing the whole of him/her self.

Scientific colleagues

The interaction with friends and colleagues is an essential activity during the agony of

hypothesis testing even the discovery itself. Close and warm personal interactions may be crucial in the resolution of the problem. These dialogues are vital and are the very essence of science *and depend on personal encounters*.

A critical approach

The best scientists are those that can ruthlessly criticise their own ideas, and strive to prove them incorrect. But the commitment described above must always be there to drive the hard work and effort.

The way

Thus the scientific method described above is deeply ingrained in the scientist and is the way, the path to truth about the material Universe. This way has been very successful, and is so valuable because error in the interpretation can be rapidly eliminated due to continuous discussion and revision and further experiment. Humility is essential in a good scientist, because he/she must value and be open to the work of others.

Scientific hypotheses which do not fail are termed theories or laws although all scientists recognise that these can be modified with further experimentation [3]. Prefers the word "habit" to law in a stimulating critique of the dogmas of modern science.

We describe below how this process actually operates in the lives of the scientists who were kind enough to contribute to this paper.

Kusel writes

My PhD supervisor was Don Northcote in Plant Biochemistry, Biochemistry Department and Tennis Court Road Cambridge University [4].

Don Northcote, as an initiation into scientific research, gave me advice which I have never forgotten. "Follow your results" was his research philosophy, and I have incorporated this in my research, and advised others to do the same. This phrase removes the rigidity from scientific investigation, and later, a friend and colleague from France, Helene Mone, exhorted us to "look, and look and look". This is similar to the "take time to look" of Barbara McClintock and the "take a closer look of Leiper himself. I like to add "make your own mistakes" and do not be frightened to make them because doing this is a potent way of investigating unknown areas.

During the research I had come across heavy staining using the Gomori histochemical technique for phosphatases, in some internal membranes of the pea root cells. This encouraged me to work on "Plant Cell Membranes" for my PhD thesis, a subject which had not been entertained by Don thus far in his laboratory. It was an exciting project and I was given complete freedom to make my own mistakes and to try out my ideas. This experience was a harsh teacher, because many things I tried did not bear fruit and I published nothing from this work. However, this sense of freedom gave me a sense of adventure, and the independence taught me to think on my own and with confidence.

During research for the PhD degree I had yearned for some practical use for my studies and had thought about studying medicine. Joseph Ndabahweje, the technician from Uganda working in Don's laboratory, suggested that I should think about working on a disease called Bilharzia or schistosomiasis as it is more usually called now. In Uganda and many other African countries, Bilharzia (schistosomiasis) is a very common disease transmitted by water snails and I had never heard of it!

In the 1960's there were no good drugs against schistosomiasis, (or bilharzia), the suffering was considerable and visits to the Pediatric ward in Khartoum Civil Hospitable was heartrending. We felt tearful compassion, especially for those very heavily infected. This was the beginning of action and discovery and the practical application of science that I had missed during University education.

We started work on the disease by establishing a snail colony in the laboratory and infecting the snails with the eggs from faces obtained from patients from the hospital.

Since the eggs were very readily available, my first studies were directed towards the nature of the structure of the egg and its mode of hatching. This research was carried out by the use of the light microscope and involved very simple observations and ideas drawn from these. One strange phenomenon in the structure of the egg, as seen under the microscope is a balloon-like body. The nature of this was completely unknown, so I attempted to reveal, by immersing the egg in solutions of increasing density of albumin, the internal structure of the balloon. I concluded that it was cellular and was the remains of the vitelline cells which are used for nourishment. I also wanted to determine how the egg hatched, since the egg shell appeared to break open at random regions, unlike the egg shell of some other worms such as the liver fluke. I examined hundreds of hatching eggs and open egg shells, and investigated number of stains to highlight the broken region. I concluded that there was no clear region which showed the initial weakness. It was also possible to initiate the hatching in dead eggs by the use of osmotic stress caused by glycerol.

All these experiments may seem very simple but for me, new to the field, they were very exciting. The observations were novel and I published the observations, my first paper ever (Kusel 1970). I received news of the successful review of this paper with news of another paper that I had submitted on the penetration of human skin by the cercariae to produce schistosomula. I recount these stories because the editor of the journal “Parasitology” took great pains to point out the Latin origin of the word schistosomulum, (originally from the Greek skistos and soma) and this gentle caring scholasticism in editorial comments (the Editor was Professor H.D. Crofton) would be most welcome in these modern times and made a lasting impression on my attitudes towards the attempts of others to publish new findings!

I made an observation when looking at the schistosomula produced during skin penetration. They showed a strong binding over their surfaces to isolated erythrocytes which had strayed into the human serum which I was using to collect the schistosomula. At this time I had visited Ron Smithers at Mill Hill and he had outlined the work they were doing there and I was able to discuss my plans. He and his group had shown that the schistosome acquires host antigens on its surface. This was in contrast with the experiments of Andre Capron and his group, who speculated that the host antigens were synthesised by the parasite. When I observed the binding of the erythrocytes to the surfaces of the schistosomula, I jumped to the conclusion that here was a possible method by which the host antigens could be transferred to the parasite! Very much later this idea was confirmed when I was working with the Smithers’ group. (I mention this, not to claim any priority, but to show that ideas can be sown as a seed by observations which have no direct relevance at the time).

Again, later work showed that Capron was also correct and that some host antigens can be synthesised by the parasite. This is often found in science where two apparently opposite views reflect the unexpected complexity of living organisms.

The host antigen hypothesis

In about 1968 Ron Smithers and Roland Terry had evidence that the schistosome tissues contained host-like molecules. At a meeting of the BSP a colleague suggested that these might be acquired from host serum. Smithers and Terry immediately set up experiments to detect the presence of host molecules on the surface of the adult parasite. Rhesus monkeys were immunised with mouse serum proteins and adult schistosomes from infected mice were transferred by a surgical procedure into the portal veins of the monkeys. Monkeys without immunisation were used as controls. After several weeks, to allow the transplanted worms to adapt, the monkeys were perfused and adult worms recovered from the portal system.

The number of worms recovered from the immunised monkeys was significantly less than those recovered from the control monkeys. This was evidence that the worms grown in the mice carried mouse macromolecules on the surface and these were detected by monkey antibodies which destroyed the worms [5].

After many experiments it was clear that the surface membrane appeared to carry the host molecules. What were these host antigens doing? Clegg, who joined Smithers in 1968, designed experiments to test the hypothesis that the host antigen acted as an immunological disguise, and obscured parasite antigens from host antibody binding. Experiments were carried out using schistosomula cultured in the presence of serum and red blood cells and there was a clear correlation between the uptake of antigens from the red blood cells and the lack of binding of anti-parasite antibodies to the cultured schistosomula.

It was very unclear as to how the host antigens were acquired. I urged John Clegg to go with me to a seminar on serum complement binding to membranes to be given by Robin Hesketh (now also an author). A valuable feature of the atmosphere in Mill Hill was that there were many seminars on all subjects, and such seminars greatly stimulated interaction between Divisions of different biological disciplines. A good principle is to go to seminars which are not directly relevant to your own research problems. Often a spark can come from what you hear! Robin was concerned with measuring the binding of complement to artificial lipid membranes. The insertion of antigens into the membrane involved incubating liposomes with the glycolipid Forsmann antigen. Antigen uptake occurred by insertion of the lipophilic tail into the membrane. This statement caused John and I to look at each other amazed and excited. This could be the way host antigens, in glycolipid form, might insert into the schistosome membrane! We had previously assumed that the host antigens were proteins. This “discovery” was greeted with excitement in the laboratory and a number of experiments were quickly designed to test the idea that the host antigens might be blood group glycolipids. Blood group glycolipids could be shown to be taken up *in vitro* by schistosomula during growth and incorporated into the surface membrane. The crux of the problem for the host antigen hypothesis was whether the recently acquired blood group substances actually prevented host immune mechanisms from damaging the parasite once it was realised that the red blood cell antigens might be blood group glycolipid substances, Owen Goldring [6], showed that glycolipids could be readily acquired from cells in culture [7]. Harnett showed a correlation between loss of red blood cell antigens from the adult worms and exposure of parasite antigens. Some scientists had shown that there was a decrease in host antibody binding to the parasite during prolonged culture even in the absence of any host molecules. Thus the changes in the surface membrane could be intrinsic and the acquisition of host antigens might be a totally separate phenomenon, based on physicochemical nature of the membrane itself! El Ridi and colleagues [8], have a very original study in which the biophysical nature of the surface is responsible for the lack of exposure of parasite antigens. They have shown that the change in metabolism of sphingomyelin in the surface membrane can account for a change in orientation of parasite membrane proteins.

The surface

The work I was doing with the skin schistosomula enabled me to examine the differences between the surfaces of the cercaria and the schistosomulum. Using simple salt solutions to inhibit the dissolution of the surfaces by acid or alkali, I was able to speculate that the surfaces were stabilised by ionic forces and salt bonds Although modern studies of the surfaces [9], have defined the proteins and their location in these surfaces, we still have no idea of how the molecules are bound together, or inserted in a lipid membrane.

In order to explore the surface structure further we tested how easily the surface could be damaged by detergents. We came across avery unexpected finding. The detergent digitonin caused the surface of the cercaria to swell and become fragile, but did not dissolve it. Digitonin was known to bind to cholesterol in membranes and so this surface seemed to partly consist of a conventional membrane. After pondering for a day or two, it suddenly struck me that it would be possible to use this treatment to isolate the membrane and perhaps use the preparation as a vaccine. I had previously been to see Ron Smithers in Mill Hill and met the gifted David Hockley, a superb electron microscopist. Professor George Nelson was visiting Khartoum the next week so I prepared surface membranes, fixed them in formaldehyde and gave them in a small tube to Professor Nelson.

David was able to produce some beautiful electron microscope sections of these surfaces and I was able

to publish our findings in a paper which was the first to describe membrane isolation. In keeping with his self-effacing humility, David did not want joint authorship although I urged it, but wished only to be thanked in the acknowledgements.

The moment of realisation that the membrane could be isolated from this observation was a moment of discovery. Many ideas about the possibilities engrained in the discovery swept over me and I was extremely excited but calm. This moment is not important in the history of schistosomiasis, because other methods have been discovered since, but to me it gave me an insight into how creative scientists and other creative workers feel at the moment of discovery. Many strands of knowing and experience were involved in the process and thus it was intensely personal and the whole of me as a person was involved in it. I left Sudan in 1970 and had the temerity to approach Smithers to see if I could work in his laboratory for the summer. He agreed! I started work and immediately noticed the atmosphere of imagination, good personal relationships full and frank discussion and hard work.

I continued the work I had initiated in the Sudan, and tried to reproduce the results I had obtained with cercariae and schistosomula, in which the surface could be isolated using saponin or digitonin. It was quickly found that saponin, especially with the addition of calcium ions, could be used to strip off the outer tegument of the adult male and female parasite and thus could be a basis for the isolation of the surface membrane. I read this as a paper (my very first!) at the Spring Meeting of the British Society for Parasitology (1970). I had prepared the talk with the help of John Clegg and Ron Smithers, but this did not prepare me for the storm of adverse questions which followed my talk. The main problem was that I claimed to have isolated the tegument of the larval and adult stages, but quite correctly one of the Electron Microscopists in the audience objected (Dr Kate Lyon in the very front seat!), that it was only the membrane that had been isolated. To call it tegument was incorrect. Another criticism was that saponin would destroy the phospholipids in the membrane itself, so the criticism hit both ways! I was chastened and resolved to be more precise in terminology and in preparation for questions in the future. I subsequently found a way to isolate the membrane by a freezing and thawing technique, which is still used to isolate membranes prior to the determination of the protein architecture of the membrane [9].

In Glasgow University the development of the research programme, I tended to follow the words of Don Northcote “follow the Results” This meant that apart from the necessity of planning for the writing of grant applications, the progress depended on intuitive processes stimulated by observation. This suited my way of working but it was puzzling to others and it did not make me a great group leader or principal investigator, because I was considerably dependent on the observations I myself, made in the laboratory. Our small group consisted of Lesley Stones, Batool Al-Adhami and Joyce Thornhill. We were joined by Janet Jones who introduced an interest in genetics and then by Billy Harnett. Later we welcomed Leda Vieira from Brazil.

My philosophy was to encourage each to have their own project and I worked away on my own ideas. Lesley was my technician although she was encouraged to work with others since we had a flexible system. Joyce Thornhill was responsible for the maintenance of the snail colony and her powers of observation were such that she developed her own research project on the laying of eggs by infected snails.

Billy Harnett writes about discovery in this way

My understanding of the excitement of discovery only really emerged during my PhD studies. I had been an undergraduate student on the BSc in Immunology course at the University of Glasgow and during the final (Senior Honours) year a major component of the course was the undertaking of a lab-based project. Fortunately, one was able to select a project from a list which is offered by members of staff and I chose to work with Dr John Kusel in the Department of Biochemistry on a project entitled “Investigation to explain non-adhesiveness of the surface membrane of adult *Schistosoma mansoni* for mouse peritoneal cells”. My interest in parasitic worms like schistosomes was a consequence of the very first lecture I attended at Glasgow: It was delivered by Dr Adrian Hopkins who described tapeworms in a highly entertaining

discourse on the gut. When I arrived at Glasgow my intention had been to specialise in Chemistry but I was so impressed by this lecture that I ended up switching to Immunology.

Undaunted by my idiosyncratic performance during my Honours project, John sounded enthusiastic when I broached the subject of a PhD. John thus submitted a grant application to the MRC but unfortunately this was unsuccessful. I thus accepted a Research Assistant position at Glasgow Royal Infirmary to work on detecting carcinoembryonic antigen by radioimmunoassay (RIA) and this also had the carrot of working towards a PhD thrown in. However, the position largely involved routine clinical immune-diagnosis and so when John informed me that a resubmitted grant application was successful, the opportunity to go back to research into the immunology of parasitic worms proved too strong a lure to resist and thus I returned to his lab after one year at the Royal Infirmary. Interestingly however, what I had learned on the subject of RIA during this year proved to be key to the research I was subsequently to undertake during the next few years.

My PhD was related to my Honours project in focusing on the schistosome surface membrane. This is a very intriguing structure, electron microscopy revealing that it was unusual, indeed unique, in consisting of a double lipid bilayer. Furthermore, the outer bilayer was known to contain molecules, which had been acquired from the parasitized host. These were largely glycolipids found on red blood cells and it was considered that they acted to disguise the schistosome from antibody-mediated immunity (the "host antigen" hypothesis, put forward by Ron Smithers and Roland Terry). The focus of my research was to investigate aspects of this hypothesis and in particular, to determine if it was possible to expose parasite antigens that could act as targets for antibody by interfering with the host antigen coat. Towards this, antibodies were already available in John's lab against both parasite and host antigens and it was possible to detect binding of these reagents by indirect immunofluorescence (IF). However, on starting to explore this system, I quickly realised that a more quantitative assay was required (the IF procedure only being semi-quantitative) and I therefore decided to use my previous experience to develop an RIA.

This proved to be more challenging than expected, in particular a great deal of work on optimisation was required, but eventually I managed to design an indirect radiolabelled antibody method (the "IRMA") that was able to quantify the binding of both antisera to the living schistosome surface using I-conjugated second antibodies. The question then was would I be able to perturb the membrane in some way to reduce expression of host antigens and at the same time promote increased expression of parasite antigens and equally importantly, would my assay be able to measure the change? Again, this took some time but eventually I was able to achieve success simply by using formaldehyde–I found that exposure of adult male worms to this reagent decreased expression of host antigens and increased exposure of parasite antigens. Within the Biochemistry Department, there was a little bench-top gamma counter, which I had virtually commandeered for my own use and I still remember clearly the feeling of exhilaration standing watching the first positive data emerge from the gamma counter's printer (~09.25 in the evening and I think it was a Tuesday….).

Following this, inspired by Mike Doenhoff who had published on the interaction between anthelmintics and the immune system, I went on to show that the key anti-schistosome drug praziquantel, whose mechanism of action wasn't fully understood, also exposed parasite antigens at the schistosome surface. This was a potentially important finding with respect to chemotherapy.

Leda Vieira writes on the topic of discoveries

Serendipity is probably the word that best describes my most successful research. I have always worked on immunology of parasitic diseases, but the roots of the Department where I did my PhD-and now work at-are in Biochemistry. Therefore, I have always had a sentimental link to the Brazilian Society for Biochemistry and Molecular Biology. In order to attend the meeting, I would send papers on teaching Biochemistry (which I teach to undergraduate courses). One year, a group of students taking a practical course in Biochemistry decided to investigate the production of reactive oxygen species by macrophages from the several knockout mice I had in my lab. They did the experiments and some interesting results

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were produced. Instead of taking my poster to the Education section, I dared and took it to the Free Radical section. There, I met two prominent researchers in the field, who criticized the poster severely. One was interested in doing some experiments with knock-out mice for the phagocyte NADPH-dependent oxidase (phox ko) infected with *Trypanosoma cruzi* and proposed that we did them together, since I had the capacity of keeping immunosuppressed mice successfully. We did these experiments in collaboration, and found that, when infected with *T. cruzi,* parasitemia is the same in wild-type and phox ko mice and so is organ parasitism. However, mortality was much higher in phox ko mice. In fact, they died as quickly due to infection as the highly susceptible interferon-γ ko mice. However, interferon- γ ko mice had a much larger parasitemia. So, phox ko mice controlled parasite growth but also died in the acute phase of the infection. What was killing them? As it turned out, it was an excess of nitric oxide and consequent low blood pressure. When present in equimolar concentrations, nitric oxide and superoxide react and produce peroxinitrite, which has no effect on the blood pressure, albeit toxic to the parasite. In phox ko mice, superoxide was not present to react with nitric oxide. Thus, nitric oxide from phagocytes that were activated to kill the parasites (hence producing nitric oxide) was active in lowering blood pressure (Santiago et al., 2012). I was subsequently invited to be a part of a National project on Redox Biology by the second researcher I met at that meeting. Since then, our lab has produced thesis and papers on the subject. Had I not gone to the Free Radical section of the meeting and had I not attracted the criticism of the two experts, I would have never entered this field, which has been indeed gratifying. Interestingly, part of my PhD thesis was also done using two techniques that were introduced to the lab by serendipity. I worked with surface vesicles of schistosomula from *Schistosoma mansoni* that were obtained because my supervisor (Professor Kusel) had unintentionally added hyperosmotic saline to the parasites (and actually *looked at them* under the microscope instead of discarding them) (Vieira et al., 1987).

Skin schistosomula

On several occasions during incubation of mechanically derived schistosomula [10], with fluorescent membrane-impermeant dextrans, we noticed that a small proportion of living undamaged schistosomula were labelled in their excretory tubules. How did the large molecules enter the schistosomula? The membrane was thought to be impermeable to proteins and dextrans. By a coincidence of observations we hastily jumped to the conclusion that the large molecules had entered through the nephridiopore. We had noticed during *in vitro* investigations with the adult worm that during damage to the surface, fluorescent dextrans and serum albumin entered the nephridopore. In other experiments, damage to the schistosomula surface gave heavy labelling in the region of the excretory region. So we were open to the idea that entry of molecules could occur by this pathway. This suggestion was resisted by many of my colleagues, vigorously so in some instances! I mention this because it is easy to see a certain path where another may exist. Eager to test this hypothesis, we transformed schistosomula by the syringe method in the presence of a variety of membrane impermeable compounds (dextrans, proteins, propidium iodide). A proportion of schistosomula showed uptake concentrated in the excretory region. All data was consistent with the nephridiopore being the route of entry. Fluorescent poly-l-lysine was localised in the excretory tubules and bladders so we felt confident that this could be the correct interpretation. But an alternative explanation not considered initially was that large molecules could enter the schistosomulum through the surface membrane itself.

We studied the uptake of large membrane impermeable compound when injected into the pathway of penetrating cercariae during passage through the skin of mice. There was massive uptake into the body of the schistosomula and not only in the excretory region.

When we repeated the experiment by injecting fluorescent dextrans and the endocytosis marker FM 143, into the region of penetration of mice 24 hours and 48 hours after infection, a similar massive uptake of dextran and of FM143 occurred. This suggested strongly that the molecules had penetrated through the surface membrane during their residence in the skin. These *in vivo* labelling patterns were not observed during *in vitro*.

We were very excited by this because it seemed that passage and migration through the skin induced a great increase in membrane activity. Could this also be true in other parasites? Perhaps trypanosomes, malaria [11] and nematodes [12], experience similar changes in their surfaces during residence in the skin.

Thus in this section we have seen that hypotheses have been suggested due to hunches and that these have sometimes led the scientist astray and to pursue work on the hypotheses even though evidence was incomplete. This is an example of resistance within the scientist himself [13].

The excretory system

The observations of Fabio Ribeiro [14], had sensitised us to "look for" phenomena involving the excretory tubules. We came across an example of this when looking for hydrogen peroxide in the adult worm using the dye Amplex Red, which liberates the highly fluorescent resorufin in the presence of hydrogen peroxide and peroxidase. To our amazement the resorufin was concentrated in the excretory tubules and ejected from the worm through the nephridiopore. Resorufin is part of a family of molecules which are recognised by the P glycoproteins which excrete drugs from drug resistant cancer cells. This led us to hypothesise that P glycoproteins were concentrated in the excretory tubules. Thus the excretory system might be a means by which the parasite excreted drugs, importantly praziquantel. This is being exhaustively studied by the laboratory of Bob [15]. Currently no concentration of P-glycoproteins has been detected in the excretory tubules, so our explanations may be too simple!

Donato Cioli writes as follows

I entered the field of parasitology through a rather uncommon door, i.e. on the basis of a rational free choice. Back in 1968 (and the date says a lot), I was one of a group of young postdocs at the Salk Institute (La Jolla, CA) who engaged in a collective discussion about the social meaning of our research and, after laborious study and lengthy consultation, decided to re-orient the contents and the goals of our work[1]. In our bold excursus of the most pressing needs of mankind, we came across a parasitic infection, schistosomiasis that affected 200 million people, was mainly prevalent in poor countries and appeared to be under-studied and–powerful motivation– was also biologically fascinating. With the naive self-confidence of our age, we thought we could contribute to some of the most pressing needs in the field (no vaccine, poor drugs and incomplete knowledge of the pathogen). As a result, four of us decided to devote our research efforts to that disease once we could establish our independent laboratories. This is what eventually Alan Sher did at NIH, Paul Knopf at Brown University, Italo Cesari in Caracas, Venezuela, and myself in Rome–a crew some jokingly labelled as "the La Jolla four".

Influenced by our previous training, most of us initially addressed the immunological aspects of the disease. At that time, the "host antigen" phenomenon described by Smithers and Terry was attracting a lot of attention and everybody's goal was to find ways to circumvent it. But at the same time, the idea of such a clever mechanism developed by the parasite almost unconsciously stimulated a sense of awe and admiration toward such a respectable enemy. I remember indulging oftentimes in a mute dialogue with those elegant, dignified, lively schistosomes I was observing at length under the microscope as if asking them to finally disclose their secrets. The dream of a vaccine was irresistibly attractive: a radical prevention, possibly a way to interrupt transmission, no more repeated treatments, no more disease. Yet the challenges were enormous: only partial and very delayed acquired immunity in humans, disappointing and controversial results in experimental animals, a baffling array of potential but ineffective antigens.

At some point, the goal of a vaccine seemed so distant in the future that maybe the disease would be sooner vanquished by other means–hopefully just by economic progress. In the meantime, disease control had made substantial progress with the introduction of safe and effective drugs, so that large numbers of people were actually experiencing significant, albeit not definitive, health benefits. The field of antiparasitic chemotherapy is filled with a number of challenging problems, a crucial one being the fact that the mechanism of action of many drugs is often unknown, which makes the development of improved medications a largely

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empirical process. As to schistosomiasis, the most significant discovery, after the elucidation of the life cycle (*e.g.,* Manson's work), has probably been the introduction of praziquantel, a drug capable of producing complete worm eradication with minimal side effects after a single oral dose. The main problem with praziquantel is the fact that, due to its success, it is practically the only available antischistosomal drug and, after decades of mass administration to millions of people, the danger of drug resistance is a serious threat.

There is another antischistosomal drug that is as safe and effective as praziquantel, *i.e.* oxamniquine, but it has been practically abandoned because–unlike praziquantel–it is effective only against *S. mansoni* and not against the two other major schistosome species (*S. haematobium* and *S. japonicum*). I had been intrigued by a very similar drug, in fact a progenitor of oxamniquine, called hycanthone, whose mechanism of action appeared uniquely approachable due to the existence of schistosome strains that were highly insensitive to the drug. The obvious idea was that understanding the nature of the difference between sensitive and resistant parasites would give a crucial clue to the drug mechanism of action. The two schistosome strains–sensitive and resistant–are remarkably stable, an immediate enticement to explore the genetic basis of their different behaviour. Among the possible ways to perform genetic crosses between schistosomes, I was particularly attracted by what appeared to be the most straightforward and cleanest approach, namely to mate a single male and a single female worm and analyse their progeny. When I was an apprentice in schistosomiasis, I had seen Ron Smithers performing his elegant worm transfers into the mesenteric veins of Rhesus monkeys and I had later adapted the technique to the size of mouse veins[2]. Thus, I resorted to this method to investigate the genetic basis of drug resistance in schistosomes.

I wouldn't have made much progress in this whole story without another fortunate circumstance, *i.e.* the precious collaboration with my long-time friend and colleague Livia Pica-Mattoccia. Together, we crossed sensitive and resistant schistosomes and from the analysis of F_1 and F_2 progeny, backcrosses and genetic complementation experiments, we got evidence that resistance was determined by a single autosomal recessive gene[3]. Discovering that schistosomes, like other metazoans and like humans, obey to good old classical Mendelian laws was not at all surprising from a scientific point of view, yet it somehow strengthened a feeling of closeness and almost of friendship with those remarkable creatures.

The fact that resistance to hycanthone and oxamniquine was a recessive trait, immediately suggested that resistant schistosomes were lacking some critical factor, probably involved in a drug activation mechanism. Indeed, biochemical experiments showed that an extract of sensitive schistosomes rendered the drug capable of covalently binding to the DNA of parasites, whereas the equivalent extract from resistant worms failed to do so. Fractionation of the worm extract showed that the activating activity had a molecular weight of about 30kDa and that the activation reaction required the presence of a sulfate donor. Together with a number of additional pieces of evidence, we came to the conclusion that antischistosomal activity was dependent on the presence of a parasite sulfotransferase[4], but we were unable to decide which one of a number of schistosome genes coding for sulfotransferases was the real protagonist. It was only with the help of cutting edge techniques of linkage mapping and crystallography provided by the collaboration with Phil LoVerde and Tim Anderson that the crucial gene (called Smp_089320) was unequivocally identified[5]. It was indeed a sulfotransferase, thus vindicating a prediction we had made decades previously and providing us with one of the most gratifying feelings in the risky life of a scientific researcher.

Now that we know the molecular picture (in fact, the atomic details) of how oxamniquine binds to the activating sulfotransferase of *S. mansoni*, there are real possibilities of designing drug analogues that would bind to the orthologous genes of *S. haematobium* and *S. japonicum*, thus providing valid alternatives-or supplements-to the exclusive use of praziquantel. Work in progress in that direction is extremely promising, thus fulfilling at least a minuscule portion of those juvenile dreams of doing research as close as possible to human needs.

Paulo Coelho writes

In the decade of 1970, Dr. Jose Rabelo Freitas (deceased in 2014), a reputed ecologist from Federal

University of Minas Gerais– Department of General Biology with many important contributions about ecology of snails genus *Biomphalaria,* transmitters of schistosomiasis mansoni in Brazil) and I were studying the susceptibility of geographical *B. tenagophila* strains from different regions in Brazil challenged with geographical strains of *Schistosoma mansoni*. Among dozens of *B. tenagophila* geographic strains we found one coming from Biological Reservoir in Taim, State of Rio Grande do Sul. This lineage was challenged with different strains of *S. mansoni* and with variable burdens of miracidia, showing always absolute resistance to infection [16]. *B. tenagophila* resistant lineage arouse in us a huge interest because this species is the second more important of genus *Biomphalaria* related to the transmission of schistosomiasis in Brazil.

A fundamental question was: Does *S. mansoni* resistance show dominant or recessive character? The cross-breeding between *B. tenagophila* Taim lineage and the *B. tenagophila* albino strain (recessive phenotype), highly susceptible to *S. mansoni*, resulted in an F1 generation totally resistant to the parasite. The F2 generation showed about 25% of susceptibility, clearly according to the classic Mendelian genetics [17].

Further studies demonstrated that the transference of hemolymph from Taim lineage to a susceptible strain resulted in a significant increase of the resistance from the receptor lineage against *S. mansoni* infection. The transplantation of the hematopoietic tissue from Taim lineage to another susceptible snail strain showed that there occurred an absolute resistance to the parasite in the receptor strain when the transplant became established. For this last experiment, we had a molecular marker represented by a 350 base pairs band of the ribosomal RNA internal transcribed spacer region (ITS), which is peculiar to the Taim lineage. This marker allowed us to evaluate the success of the transplantation (the presence of haemocytes with the marker of 350bp demonstrated the success of hematopoietic tissue transplant) [18].

Other studies demonstrated that internal defense system of Taim lineage is responsible for Taim lineage resistance to *S. mansoni*. This mechanism is hugely efficient eliminating the parasite rapidly in *in vitro* and *in vivo* experiments through the confrontation of hematopoietic cells from Taim lineage with *in vitro* transformed *S. mansoni* sporocysts. In both cases, the sporocyst death occurs in a few hours (*in vivo*: less than 6 hours). It could be inferred that the absolute resistance of Taim lineage to *S. mansoni* is not due a simple incompatibility between parasite and invertebrate host but related to a sophisticated mechanism linked to the internal defense system of snail [19].

After decades of studies, these results allowed us to test the hypothesis that the introduction of this Taim lineage into endemic areas (in which *B. tenagophila* is known to be the transmitter of schistosomiasis [20], might decrease the transmission of the disease. Therefore, we obtained all environmental licenses for field intervention. The breeding of Taim lineage in a mass scale is well stablished and has been used to produce many thousands of snails in the city of Bananal, State of Sao Paulo, in collaboration with Dr. Engels Maciel and support of SUCEN (Superintendência de Controle de Endemias de São Paulo) [21]. *B. tenagophila* Lineage Taim snails were introduced into the locality of Herivelton Martins stream that contained local snails with an average susceptibility of 30% before introduction of those of Taim lineage. After 15 months of the introduction, only 2.1% of the offspring snails, that resulted from crossbreeding between Lineage Taim introduced snails and local snails, were infected. Furthermore, it was observed that there was a strong correlation between the presence of the *B. tenagophila* Taim molecular marker in the offspring and resistance to infection by *S. mansoni*. Studies conducted in the area showed that during the occurrence of floods, other tributaries of the watershed were connected with Herivelton Martins stream triggering the reintroduction of susceptible local snails in the ecosystem. Susceptibility tests in these condition showed 15% of susceptibility of young snails and a reduction of the 350bp molecular marker in this population. New introductions will be conducted in the Herivelton Martins stream and in new susceptible *B. tenagophila* habitats of this region. These results have shown the difficulties and complexity of studies in natural environment.

Recent studies of hematopoietic cell cultures (hemocytes) are been conducted as well as molecular

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studies using bioinformatics tools, microRNA sequencing, transcriptome and genome sequencing of *B. tenagophila* Taim lineage aiming the identification and isolation of the principal gene involved in the resistance. An ambitious purpose is to create a *B. glabrata* (most important species for schistosomiasis transmission in Brazil) resistant transgenic strain using transfection techniques to transfer the gene (s) of Taim lineage to *B. glabrata*. If this transgenic approach in *B. glabrata* works, the same approach could be used in other *Biomphalaria* species in the African continent, then all susceptible to *S. mansoni*.

Alan Wilson writes

I have a vivid memory of my first encounter with schistosomes as a student at Imperial College (IC), when Dr John Jewsbury from the Natural History Museum, dissected out adult worms from the hepatic portal vein a mouse infected with *Schistosoma mansoni*. Little did I realise that event would shape my subsequent career. In 1962 I was offered a PhD studentship at IC with a free choice of topic and picked the liver fluke *Fasciola hepatica*, receiving external guidance from Dr Ollerenshaw at the Central Veterinary Laboratories in Weybridge. A lecture by Francis Crick on the genetic code convinced me that there was a lot of exciting work at the molecular end of biology about which I was woefully uninformed. In the then time-honoured way, I remedied that deficit at "night school", a three-year Diploma in Biochemistry, at Chelsea College. In 1965 I took the train north to a building site called the new University of York and a post as Lecturer; I had complete freedom to develop both my undergraduate teaching and my own research inclinations. At York I built up a research group probably amounting over the years to ~90 post-graduates, post-doctoral fellows and visiting researchers, not forgetting the yearly intake of project students, ~150 in total. It would be invidious to single out individuals but their overall contributions were immense. Two colleagues, Dr Patricia Coulson (1972) and Dr Adrian Mountford (1985), eventually became permanent members of the Schistosomisis Research Group on University posts.

In the 1960s electron microscopy was the cutting edge technique, and whilst still a PhD student at IC, with the help from Dr Mary Morris, I obtained images of the liver fluke egg as part of a study of its hatching mechanism, using a microscope in the Department of Electrical Engineering. Fortunately the York Biology Department decided to invest in a new-fangled electron microscope suite, allowing me to explore the ultrastructure first of *Fasciola* and later of *Schistosoma*. In 1970, with help from Dr Ron Smithers at the National Institute of Medical Research in London, we set up the *S. mansoni* life cycle and I got my first MRC grant to investigate the schistosome tegument.

Papers on tegument ultrastructure and function followed in 1974, documenting the existence of a secreted "membranocalyx" overlying the plasma membrane of the tegument syncytium, so initiating a lifelong fascination with immune evasion at this parasite-host interface. A meeting with Darwin Murrell in Cairo in led to my spending a sabbatical in his group at the Naval Medical Research Institute (NMRI) in Bethesda, Maryland, in 1976 and another short spell in 1979. These visits ultimately led to studies on tegument surface labelling and membranocalyx turnover. We also developed an existing freeze-thaw procedure to produce highly enriched tegument surface membranes (1983) but ways to characterise the surface molecular composition were still decades away.

Around 1975 we began to research the kinetics and route of migration that schistosome larvae took from the skin to the portal system. It was known that schistosomes left the skin and travelled to the lungs in the vasculature before somehow materialising in the portal tract. Our hypothesis that the schistosomula might take an entirely intravascular route, first elongating to crawl through vascular beds, was met with some incredulity. However, the labelling of cercariae with Se Seleno-methionine and their subsequent detection in compressed mouse tissues by autoradiography, some of the work carried out in collaboration with Drs Dean and Mangold at NMRI in 1983-1984, decisively settled the question of route and rate of parasite transit to the liver. The ramifications of this work continue to the present.

Concomitant immunity and death of invading parasites in the skin were the zeitgeist of the late 1970s and early 1980s. Based initially on experiments with rhesus macaques, it had been suggested that primary

worm burdens persisted, yet at the same time protected the infected host against invasion by further larvae. The paradigm was quickly extended to humans and to other laboratory hosts. The mechanism for parasite killing *in vitro* was elegantly dissected by several groups, who showed that newly transformed skin-stage schistosomula were highly susceptible to antibody-dependent cellular cytoxicity mechanisms.

Very slowly over a period of years the story began to unravel. A vital piece of evidence was always lacking, namely the demonstration of dead and dying parasites in the skin of hosts with concomitant immunity. A rejected manuscript describing our failure to find such dead parasites in a histopathological study of skin invasion in "immune" mice, still resides in my filing cabinet! Another problem was that concomitant immunity, in mice at least, did not develop after infection with cercariae of one sex only, suggesting that it was associated with egg-induced pathology. Given our new knowledge on the kinetics and route of migration from skin to portal system, there seemed a very obvious explanation. The deposition of eggs in the liver was known to cause portacaval shunting. We reasoned that the shunts would provide a highway for newly arrived larvae to exit the portal system. With funds from the MRC we were able in the mid1980s to confirm the "leaky liver" hypothesis as the cause of "immunity" in mice with a chronic infection. The parasite tracking studies mentioned above, finally demolished the concept of concomitant immunity in the chronic mouse model.

Building on our recently acquired knowledge of the tegument, migration and immunity, we turned to the use of attenuated cercariae as the basis for a schistosome vaccine. I had already worked at NMRI alongside Drs Minard and Dean, leading exponents of the model, as well as the people working with irradiated malaria sporozoites. The protective immunity induced in mice by exposure to attenuated cercariae appeared to provide an ideal system not associated with overt pathology. Collaboration with Dr Quentin Bickle (LSHTM) got us started with experiments, and led to the first quantitative demonstration that challenge parasites in vaccinated mice left the skin and migrated at least as far as the lungs; there they attracted effector foci of leucocytes whilst migrating along pulmonary blood vessels. Dr Brian Dixon in Leeds University gave us access to his Cobalt source and there followed a long period during which we characterised the mechanisms elicited in mice by radiation-attenuated cercariae and defined the underlying immunological processes leading to challenge elimination–in a nutshell, blocked migration not cytotoxic killing. The mediating antigens, however, remained elusive.

The relevance of the laboratory mouse to human schistosomiasis has often been disparaged. With that in mind, between 1996 and 2006, we took the irradiated vaccine model into primates, with funding from the European Commission. This involved collaborations with groups in Leiden, The Netherlands (Prof Deelder and Dr Van Dam), the Biomedical Primate Research Centre, Rijswijk (Drs Thomas and Langermans) and Kenya (Drs Reid, Yole, Kariuki and Farah) at the Institute of Primate Research, Nairobi. The outcome of these experiments was the demonstration that baboons, vervet monkeys and chimpanzees could all be protected against a live cercarial challenge by the irradiated cercarial vaccine. With multiple vaccinations, levels of protection>80% were achieved and although this protection waned with time, it was still >50% after three months. These data provide a sound basis for believing that a schistosome vaccine for human use is feasible, in spite of the fact that adult worms can reside for many years in the portal tract.

The 1980s were the golden decade for schistosome vaccines. Monoclonal antibodies became popular as a potential short-cut to identify vaccine candidates, while recombinant DNA technology made feasible both the rapid screening of immune sera for targets, and the production of the specific proteins in quantity. The upshot was that several groups published data on their favoured candidates. Such was the fervour that around 1990, the TDR division at WHO, Geneva sponsored independent vaccine trials to identify the most promising candidate to take forward to humans; the York group was one of two laboratories invited to conduct the trials. In the event, six candidates were submitted, only one of which was a membrane protein; the other five were either cytosolic or cytoskeletal in origin. Suffice it to say that in neither test laboratory did any candidate attain the benchmark 40% protection deemed essential for further development.

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Nevertheless, the ramifications of this episode linger right up to the present day, with two of those original candidates still being talked about (and trialled).

Whilst work with individual candidates has continued, there has been a slow realisation that for a schistosome vaccine to succeed, the target antigens must be accessible to the immune system in the live worm. This reorientation of approach has led the drive to characterise the parasite-host interface at a molecular level in penetrating cercariae, migrating schistosomula, and adult worms to identify the putative targets of immune attack, now a feasible goal due to the rapid advances in 'omics'. The cloning of schistosome cDNAs got off slow start with only a few hundred sequences deposited on Genbank after a decade of work, almost all encoding abundant and immunogenic internal proteins. The major effort to increase coverage was spearheaded by Brasilian researchers, (Dr Franco in Belo Horizonte and Prof Verjovski in Sao Paulo). Thanks to hosting numerous Brasilian researchers over the years, we became involved in these EST sequencing projects at an early stage and York researchers spent significant periods in those cities. The culmination of this collaboration was the publication of the *S. mansoni* transcriptome in Nature Genetics in 2003, based on the analysis of 125,000 ESTs; at the time this seemed an enormous dataset. Subsequently we were also involved in the *S. mansoni* genome sequencing project with Drs Ivens and Berriman at the Wellcome Trust Sanger Institute and Drs LoVerde and El-Sayed in the USA. This exciting schistosome community effort resulted in the publication of the draft genome for *S. mansoni* in Nature in 2009.

Around 2000 I attended a meeting in London where Dr Mark Blaxter remarked that no-one had yet used MALDI ToF on a helminth parasite. Never was a casual remark so opportune. Back in York, I asked Dr Peter Ashton about the technique. He came back with the full title "Matrix-Assisted Laser Desorption/Ionisation Time of Flight" mass spectrometry, to identify proteins in gels, and the news that we had free access to the latest instrument. We had used the O'Farrell method to separate tegument proteins in two dimensions on gels in the early 1980s but no simple method then existed to identify the many spots. Access to the Brazilian ESTs and a mass spectrometer led in 2004 to the first use of proteomics on a helminth to quantify the major constituents of four schistosome antigen preparations so beloved of immunologists. The establishment of a Proteomics laboratory in York meant we were well placed to perform compositional analyses of the cercarial secretions, the tegument surface, the vomitus regurgitated by adults and the secretions of live eggs. These are the major sources of protein input from the parasite into the host body. However, the composition of the membranocalyx has proved particularly intractable due to the lack of a robust method for its enrichment; this remains the key to understanding immune evasion in the bloodstream.

Returning to the work on schistosomes in primates, I had often wondered if the "concomitant immunity" displayed by rhesus macaques might be the result of portal caval shunting. (We had already identified shunting as the cause of "immunity" in *Rattus rattus*, and in the 129 strain mouse). The opportunity to test this hypothesis was provided by a small EC grant that allowed us to infect six animals and then after about 20 weeks, to measure porta-caval shunting and recover the worms. Shunting proved minimal in five monkeys but serendipity took a hand when Patricia Coulson noticed that the recovered worms were pallid, not the usual black colour. This opened up a whole new avenue of research that continues to the present. We showed that the surviving worms had stopped feeding and were slowly starving to death–we termed this a 'self-cure' process. Initially we identified antibody reactivities with gut and tegument proteins, but another twist was added to the story by our collaborators in China. In the mid-2000s Prof Liu and Dr Cao from the National Institute of Parasitic Diseases in Shanghai has visited York to learn proteomic approaches. In 2011, their colleague Dr Xiao Hong Li obtained funds to perform a rhesus macaque experiment with *S. japonicum*, but this time we were forewarned about what to expect. The same pattern of worm starvation and death was observed.

This work led us to focus on the neglected schistosome esophagus as a potential source of antigens, with the realisation that is was much more than a food-conducting tube. Dr Li spent 2013 in York characterising the anterior and posterior esophageal glands and she showed that their secretions initiated blood processing. With the arrival of cheap sequencing technologies, we have now identified about 40 genes

encoding secreted proteins, differentially expressed in the glands of both *S. mansoni* and *S. japonicum*; these proteins provide an entirely new cohort of vaccine candidates. Over the same period, the Brasilian 'Science without Borders' and 'Newton' programmes have maintained collaborations with researchers in Ouro Preto (Dr Castro Borges), Sao Paulo (Dr Leite & Prof Verjovski), Sao Carlos (Dr DeMarco) and Salvador (Dr Farias) and with Dr Ashton in York. These studies are aimed at linking our new knowledge on the exposed and secreted proteins of the parasite-host interface with the protective immune responses developed by rodents and primates exposed to irradiated cercariae and also in finding the targets of the self-cure process in rhesus macaques. Will a schistosome vaccine emerge in the near future? A note of caution about vaccine testing in mice was sounded in our most recent publication. In our rodent experiments we always left five-week gap between the last exposure to attenuated cercariae and the challenge with normal cercariae. The point was to allow any responses elicited by the vaccine to return to baseline before the challenge parasites entered the host. In single antigen trials that gap has shrunk, usually to two weeks and sometimes to as little as ten days as more internal, even nuclear, proteins have been proposed as candidates. We have suggested that "protection" in such circumstances results from the diversion of migrating schistosomula into alveoli during migration not as the result of acquired immune responses, but as a bystander effect of vaccination. The wily schistosome still has the upper hand.

Dan Colley writes

These are discoveries about one's self and about others around you and for me they have laid the foundations for everything else I have subsequently done (or not done) in the realm of what might be called scientific discoveries. Many of these personal discoveries came early in my scientific career, but many of them also "appeared" along the way and continue to pop-up at the age of 73.

The first of these underlying personal discoveries occurred in my first year in graduate school during a laboratory rotation with Dr. Cliff E. Dowell, working on the bacteriophage φX174 and *Escherichia coli*. The scientific discovery had to do with making *E. coli* a temperature-sensitive host for the virus and while that was satisfying, the discovery I am describing here was the discovery that generating my own data, thinking about what those data meant, analyzing those data with the help of Dr. Dowell, presenting those findings at a national ASM meeting and publishing those findings felt really, really good. The discovery was realizing that I could do something original (obviously with the mentorship of someone who really knew what they were doing), think through the process and come up with something others saw as worthwhile. That was the discovery–and it felt good! [22].

One of the first things that I discovered in schistosomiasis was that eosinophils are beautiful and alluring. Then I learned that they are also a cruel mistress. I studied eosinophils because they were there, nobody knew what they did and they were beautiful. They were a cell-type in need of a function, they hung out in and around schistosome egg-induced granulomas (the primary object of my early studies), and they were beautiful. I worked out a way collect a lot of them and to measure their in vitro migration. This led me to describe a new lymphokine (now called cytokines) that I called Eosinophil Stimulation Promoter (ESP). It was an age of discovery for cytokines and if you found one you named it for the functional situation in which you found it. It was long before the term interleukin and lots of numbers came into vogue. My lymphokine made eosinophils crawl out of agarose droplets in 96 well plates, hence the name. Plus, I liked confusing it with Extra-Sensory Perception (ESP). Some of my friends even said it was appropriate because it was found in the "medium". The name ESP stuck in the literature, that is, until one of my graduate students (W. Evan Secor) proved in 1990 that there was no ESP. Evan meticulously showed it was really a synergistic combination of IL-5 and GM-CSF produced by lymphocytes on antigenic stimulation. So my claim to fame for discovering a cytokine bit the dust and my heart was broken once again by eosinophils. Nevertheless, my laboratory studied them for a long time–during periods when they went in and out of favor. My joy in seeing them in exudates or tissue sections never diminished and while we still do not really know what they do, I don't think it ever will [23,24].

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Another early discovery in my career was that sometimes you have a theory that leads to correlative data to support it, but it is really exciting when you develop direct evidence that something active is happening in a way that you envisioned it. Here I am talking about granuloma modulation-which my laboratory studied for many years in many ways. While we did a lot of studies and published a lot of papers about them with interesting findings, it was one of the earliest that was the most exciting for me. It was when we did adoptive transfers of spleen or lymph node cells from mice chronically infected with *Schistosoma mansoni* (when newly formed anti-egg granulomas are smaller than earlier during infection) into mice with early (6 week) infections and then looked two weeks later when granulomas would usually be maximum in size. The hypothesis was that the diminution of newly formed granulomas in chronically infected mice was due to immunoregulation mediated by lymphocytes (then called suppressor T cells). The data showed that the adoptively transferred cells from chronically infected mice modulated the normally maximal granuloma formation in mice with early infections and the granulomas were smaller–like those in mice with chronic infections. This then opened the door to many, many more experiments to better define the systems and how they worked, but it was this initial discovery that was so exciting to a young investigator establishing himself in a new field [25]. Another fun discovery was instigated by a need to turn cercariae into schistosomules, i.e., the infectious form of schistosomes into the larval form that occurs immediately after the infectious form penetrates the skin. When cercariae penetrate the skin of a mammalian host they do so with a flood of enzymes and wildly active whipping around of their tails, which break off, leaving their heads (or bodies) to penetrate and become schistosomules. A lot of interesting biology is involved in this process that instantaneously changes a water-tolerant animal into a saline-tolerant animal, but that was not my problem at the time. My problem was that I wanted to use schistosomules for some experiments and I need a way to make them from cercariae without the skin penetration part. I reasoned that maybe it was the breaking off of the tail that triggered the ability of the head/body of the cercariae to turn into schistosomules. I then thought that shearing forces might make this "tail breakage" happen without too much trauma to the head/body. I took some cercariae (shed from infected snails) and ran them up and down through a 22 gauge needle attached to a 10mL syringe. It broke off the tails and then if I put the heads/bodies into saline-base culture media at 37C over-night, the head/bodies developed the characteristics of schistosomules.

This was really exciting and with a graduate student (Stephen K. Wikel) we went on to show this in more detail and published it in Experimental Parasitology–one of my very few real parasitology publications. It was exciting and fun. This story also has two other fun parts. I chose Experimental Parasitology as the place I wanted to publish this because the editor (David Lincicome) was married to the "Queen of Cercariae and Schistosomules," Peg Stirewalt. I knew that Peg would review it and the manuscript never make it to a publication unless Peg was convinced. Later Peg told me that when David gave it to her to review she immediately went in to her laboratory and reproduced the finding–22 gauge needle and all–and then recommended its publication. I found that really, really cool. The other thing about this discovery is that someone else, at the same time, discovered a different way to make schistosomules, by vortexing cercariae to make them lose their tails. The person who did this is a very good friend of mine from Brazil, Juarez Ramalho-Pinto. Without knowing it we published our two findings separately and at about the same time. About 40 years later (February 2016) I was in a meeting and heard two presentations that still used both of these methods (22 gauge needles and vortex mixers) to good effects to make schistosomules. Discovering something that is still useful decades later, that's really fun and rewarding! [10].

Starting in 1975 and continuing to this day I discovered that you can do real immunology with real people. This started in St. Lucia, then Brazil and Egypt and now continues in Kenya. Maybe the most amazing part of this discovery was that immune responses by people infected with *S. mansoni* in all of these places are relatively reproducible from place to place, if your account for the person's longevity of infection and the clinical stage of their infection. This was really a discovery for me and still amazes me, although I suppose it should not. The other, not so great discovery of working with human immunology was that you are pretty much stuck doing correlative studies rather than mechanistic studies. This is, of course, because you cannot

"experiment" with people in the same way you can manipulate or control mice or other experimental animals. Once you realize this difference it is fine, but it does take discovering or you will be frustrated. This means it is very difficult to prove something, but rather you settle for correlating outcomes to epidemiologic and clinical situations. It was also a major discovery for me to find that pregnant women impact the immune responses and immune repertoires of their babies–and the same is true with dams and their offspring in mice. This is something that I believe needs much more investigative attention, but is not something that I will eventually figure out. It will be something exciting for others to solve [26,27].

My minor adventures in investigating immune responses in human while our laboratory was studying Chagas disease is was also an exciting thing to show that there is *T. cruzi* DNA in the hearts from people who had cardiomyopathy and had died due to their Chagas disease. Until then most, but not all, investigators only supposed this was true. However, Elizabeth Jones, a Vanderbilt medical student who took a year off to work on this with us in Uberaba, Minas Gerais and Brazil was the one who finally actually proved it was true. That was exciting for both the finding and that it was Elizabeth who did it. After all, Carlos Chagas was a medical student when he discovered the entire life cycle of *T. cruzi*, so it was fitting and fun that Elizabeth Jones, another medical student, should actually demonstrate *T. cruzi* DNA in heart lesions of those with Chagas disease [28].

Back in the world of schistosomiasis, now collaborating with Evan Secor and Diana Karanja (of the Kenya Medical Research Institute, KEMRI) in Kisumu Kenya we did a longitudinal series of studies on men that were occupationally exposed to getting infected with *S. mansoni* as they washed cars or harvested sand in Lake Victoria.

With the help of these men in Kisumu, we discovered something that, in that correlative way of human data, provided additional evidence that excretion of *S. mansoni* eggs across the gut wall is facilitated by an intact CD4 immune system. This was a particularly nice thing for me to find because it was in support of a friend of mine's (Raymond T Daminan's) much earlier prediction. Ray based his hypothesis on morphologic evidence and a keen sense of the life of schistosomes. Others had also provided some evidence of this from mouse studies where you could take away the immune system of mice and see that infected, immunodeficient mice did not excrete eggs and giving them back immune cells allowed them to then excrete eggs. However we could not do that in people so we studied those unfortunate enough to have both schistosomiasis and HIV infection. It turned out that people who had the same levels of worm antigen in their circulation or their urine, meaning they had approximately the same number of worms, excreted lower numbers of eggs if they had lower numbers of CD4 lymphocytes, i.e., were more immunodeficient. We interpreted this as showing what Ray had predicted and Mike Doenhoff and others had shown in mice, namely that schistosomes cleverly co-opt their mammalian host's immune system to complete an essential part of their life cycle, getting their eggs out into the environment where they could hatch and infect snails. It was exciting to find this and gratifying to see that the predictions and experimental findings of friends had a basis for the human situation [29].

Another exciting finding in human immunology was when we, in Kisumu, provided evidence that over time and after many infections, treatments and reinfections for *S. mansoni* some people developed what we interpreted as increased resistance to reinfection. What we actually saw was that men who were occupationally exposed to high risks of reinfection, if treated every time they got infected, would, after 8 or 9 treatments and reinfection, develop longer and longer times of exposure until their next reinfection. This was paralleled by an increase in their IgE antibodies against worm antigens and we think this is evidence that repeated treatment and reinfection can lead to immune mechanisms that help kill off incoming reinfecting cercariae. This has always been a very controversial area in human schistosomiasis so finding what we believe is evidence of the development of resistance is significant. Of course continually treating and getting reinfections is a lousy way to think about vaccinating someone, but it could be taken as evidence that someday vaccine development might be achievable–given the right antigen(s) and the right adjuvant(s).

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Someday. In any case, when the data from this 5 year, very laborious study were finally analysed we were pretty excited at the finding [30].

In the realm of public health immunology and in the setting of a consortium that I was asked to put together for the Bill and Melinda Gates Foundation called the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) my colleagues and I evaluated a new diagnostic or mapping assay for schistosomiasis mansoni called the Point-of-Care Circulating Cathodic Antigen assay (POC-CCA). This assay is done on urine instead of faces (which makes the specimen much easier to get and much less smelly to handle) and it can be done in the field at the time of specimen collection. Basically, it is a lateral flow cassette assay like a pregnancy test but for whether you have *S. mansoni* infection. The standard test for this is called the Kato-Katz test and has been used effectively for over 40 years. But it is insensitive and requires feces, a microscope and a well-trained technician. And it stinks. SCORE funded investigators in 5 different African countries to test the POC-CCA in parallel with the Kato-Katz to see how it would do. It did very well and it was more sensitive than the Kato-Katz, i.e., it found more cases of schistosomiasis [31].

This is a good news-bad news situation. If there are more cases you certainly want to find them, but if you find more cases it means controlling and getting rid of schistosomiasis is going to be harder than once thought. Also, there is, with any new assay that appears more sensitive than the old standby assay, the question of specificity, i.e., is it picking up people who are not really infected? SCORE has done several more studies of the comparisons between Kato-Katz and the POC-CCA. It looks like it really is more sensitive and there really is more schistosomiasis than we thought. Maybe there is a lot more. Well that is not good news and not a reason to be excited and happy. However, it is a reason to be excited and realistic. If there is more we need to know that and up our game. How to do that is the next question. It is probably true that there is a lot more out there, but it is probably also true that many of those undetected by the Kato-Katz but detected by the POC-CCA have low levels of infection and therefore low levels of egg output. So what does that mean? Well it means we may need to make some challenging public health decisions about how important those low level infections are and what to do about them. It may boil down to what is your end goal–control or elimination? This is again part of that difficult excitement. It is a challenge and it is a meaningful challenge that has consequences for people and governments. It is all part of doing research that finds meaningful answers and thus pushes new frontiers in what we think are important ways.

I have also discovered over the course of more than 50 years of doing research for a living that the techniques and tools available change rapidly. This is great, but I have also learned that the main challenge is to ask real and appropriate questions using whatever tools are available in the time and place you are doing your research. When I started as a graduate student in immunology we did not know about T lymphocytes. Then in the major publication from my postdoctoral studies we called them “so-called T lymphocytes”. In my early faculty years we identified them by Sheep Erythrocyte Rosettes and then CD3-postive lymphocytes and found that there were subsets of T lymphocytes many subsets. Still, no matter how you identify them, find them, expand them or delete them, what really counts is whether you ask a meaningful question of them.

I have graduated 18 PhD students and mentored many more. Training each and every one of them has lead me to another discovery. It will be the last one that I will write about in this overly long discourse. Graduate students from the USA, Brazil and Kenya, and I assume everywhere else, become real scientists when they have their own data upon which to cogitate, and not before. It is, actually, quite a simple discovery. You can guide graduate students through experiments, simple or complex, but it is only after they have amassed sufficient data to allow them to think about what it means and what it does not mean that they are truly engaged in the scientific endeavour. I think this is fascinating and exciting. Once I discovered this I stopped trying to force them to think deeply about the whys and wherefores of their projects, and simply let it happen when the data started to flow. Obviously some nudging at this point can be helpful, but it is one of the most joyous discoveries that I have known-watching that happen to one of my students, and knowing that it happens all over again for me, every time the data starts to flow. It means I am very fortunate.

Andreas ruppel writes

Science is exciting otherwise one should chose a different career. I was very excited from the beginning of my University study, but then felt that this is not enough. Instead of "playing chess" (at that time my work on bacteriophage genetics gave me that feeling), I wanted to contribute to solving real problems. I was extremely lucky to be accepted as doctoral student by Donato Cioli at the National Research Council in Rome, Italy. He was a source of inspiration and patient support on my search for what I considered meaningful science. At the end of my doctoral work, science continued to be exciting, but its meaning became rather elusive. I had worked for the aim to contribute to a vaccine, which was a very fashionable idea already in the early 70's. Yet, despite many eminent scientists following that road, no one really hit the ground with something being protective against an infection with schistosome larvae (the cercariae).

I was too much focused, then, on the newly emerging field of immunology to realize the importance of the very successful work with a highly protective irradiated cercariae "vaccine" being done with bovines by Majid and coworkers with *Schistosoma bovis* and Hsü and coworkers on *S. japonicum*, not to speak of an irradiated larvae vaccine against dog hook worms, which (to my knowledge) was the first protective vaccine ever already in the 60's [32]. My own focus turned in the early 80's to the interaction of the complement system with schistosomes, being given the chance to set up a laboratory in Heidelberg, Germany, thanks to Ursula Rother (a leading German "complementologist") and Hans-Jochen Diesfeld (one of the then very few German scientists in tropical medicine, adventuring in thoughts of parasite immunology). The complement system was then high on the agenda and extrapolations from the bactericidal to an anticipated parasiticidal activity appeared as being exciting for a number of researchers in schistosome immunology.

As a young newcomer in this field and being somewhat isolated in Germany, I felt particularly glad and thankful for support by several schistosome researchers which I got to know at the Spring Meetings of the British Society of Parasitology and who responded to my great need for scientifically knowledgeable discussion partners. With some of them (John Kusel and Mike Doenhoff) I was privileged later to join in research consortia. They were among my key teachers who made me realize how important it is to share our professional expertise with young scientists in search for their future. Diane McLaren, author of the memorable yellow book on "the schistosome surface in relation to immunity" had the highest impact in the early 80ies on my scientific search, research and fascination by becoming my first international collaboration partner. Our paper in the European Journal of Immunology [33], was the very best result that could have happened to me. It gave me confidence, I was happy and proud – and we reached a relevant conclusion: the complement system is *not* the searched-for magic immunological factor killing the invading schistosome larvae soon after infection. Schistosomes simply do not bother about an immune mechanism which is directed against bacteria.

While I was busy studying the complement system to reach this important negative conclusion, which was scientifically exciting by not really glorious, monoclonal antibodies were invented and molecular biology was born. It was time to join the new trend, in particular, since in Germany at that time both technological revolutions had not yet found their way into parasite immunology. Heidelberg University provided a supportive and fascinating scientific environment, for which nowadays the terms of "center of excellence", "light house" or the like would be used. So, in cooperation with colleagues in the Institute of Immunology and the Center of Molecular Biology, we got our monoclonal antibodies [34] and we got the interesting genes cloned. It looked finally as leading to a solution of a real problem, as I had wished since 10 years earlier: This was again fascinating science but had the additional perceived potential to contribute to specific and sensitive serodiagnosis of schistosomiasis. Our monoclonal antibody met all requirements, at that time, for specificity and sensitivity, the target antigen was sequence-identified as a cysteine proteinase and its origin from the schistosome gut placed it exactly in the center of the scientific discussion of diagnostic antigens. Although this remained formally unpublished, in 1986 I performed Western blots in a village in Mali using heat-stable reagents and such basic equipment that, with the help of some curious young boys, we could produce diagnostic reactions on a village market place. Today we would call it point of care-testing.

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Diagnostics and vaccines both turned not only molecular, latest by the early 90's, but also searched for defined antigens. "Defined" meant cloned, recombinant molecules and by implication these were individual proteins. In reality, these were single proteins, widely believed to be able to do "the job", i.e. provide sensitive and specific diagnostics or immunogenic and protective vaccines. The concept that a defined vaccine (or a cocktail of such) would be able to confer protection against a schistosome infection was so much dominant that it assumed a character of dogma. At least innumerable publications concluded that the respective gene of the respective publication represented a "candidate antigen" with either diagnostic or protective "potential". I was no exception to this run for the golden bullet. Yet we have to accept–on a more modest view–that viruses with their very few genes and bacteria with plenty more, are not good "models" for schistosomes with their thousands of genes. The vaccine candidates of the 90's, which had an international standing, all failed to reach the "stated goal of consistent induction of 40% protection or better" [35]. Antigens discovered and promoted in my own group [36], were no exception to this gloriously failed rush to the "poor men's vaccine".

"Crude antigens" lacked the shine of molecular techniques, the belief in defined molecules and also lacked scientific attractivity in the sense of "advanced technology". Yet, crude antigens formed the first ever successful vaccine against a helminth (the dog hook worm), were effective in the early irradiated vaccines with Cobalt- irradiated *S. bovis* cercariae (irradiation performed in a central facility with Co source), cryopreserved (and thus transportable) schistosomula and UV irradiated cercariae (irradiated at the farmers place just before used for immunization [37]. This line of efficient vaccines remained poorly accepted and did not result in an applicable vaccine.

Defined antigens recently made their way in to specific and sensitive tests, even apt as point of care diagnostics. The antigens originated, not surprisingly in retrospective, from the schistosome gut, where they are produced "en masse" during the digestive process of schistosomes. Their early and massive production explains their advantage in the use for diagnostics over many other "diagnostic antigens". Retrospectively, we may not have pushed hard enough by a sustained flow of publications to promote the proteins identified in my group 30 years ago as diagnostic antigens. However, other antigens made it to the point-of care-diagnostics; and this is the really positive aspect of an international search for suitable diagnostic antigens.

The excitement of science made me enter schistosomiasis research and was a factor to keep me in this field. Our research in schistosomiasis was even illustrated as "giants" among the German co-authorship networks in Neglected Tropical Diseases [38]. This flatters a scientist's ego. Yet this does by no means imply any substantial contribution to towards the goals that made me enter schistosomiasis research in 1972. In 2009, Hotez and Fenwick reported the "alarming finding that fewer than 5% of the world´s people with schistosomiasis are today receiving praziquantel that costs as little as 8 cents per tablet". If I were to start research on schistosomiasis again, after over 40 years, today I would consult the practical guide on Implementation Research in Health [39] and ask what I now consider the most relevant question: "Why do we not implement, what we already know"?

Mike Doenhoff writes

I began my scientific life was as a theoretical immunologist and in 1970 obtained a PhD for experimental work on the lymphocyte system of mice performed in Tony Davies' lab at the Institute of Cancer Research in London. The 1960s were exhilarating times for cellular immunology and Davies and his colleagues, working on thymus-grafted chimaeric mice, made the important discoveries that the thymus seeded cells to peripheral lymphoid organs and that these cells divided in response to antigenic stimulation, but that they did not produce antibody [40]. My contribution was also heavily reliant on the thymus-grafted chimera and showed that the considerable number of mouse blood lymphocytes that divided in response to phytohaemagglutinin-stimulation *in vitro* were all thymus-derived, i.e., T cells [41].

After post-doc fellowships with Tony Davies, in 1975 I was offered a job working on schistosomiasis at the London School of Hygiene and Tropical Medicine (LSHTM). Mice immunosuppressed by T-cell

deprivation had been fundamental to our work in the Davies lab and while waiting for refurbished labs to be kitted out at LSHTM's Winches Farm field station in St Albans we began investigating the host/parasite relationship of *S. mansoni* in normal and T cell-deprived mice. An early observation, requiring very little in the way of sophisticated laboratory equipment, was unexpected–T cell-deprived mice excreted far fewer eggs in their faeces than normal mice [42,43]. We also showed that the *S. mansoni*-infected deprived mice suffered from a severe hepatotoxicity reaction [44], essentially the same observation as [45,46], had made earlier in athymic nude mice.

We began a search for the factors responsible for the apparent immune-dependence of schistosome egg excretion and the severe hepatotoxicity reaction in immunosuppressed mice. Passive transfer of antibodies enhanced the rate of egg excretion in the immunosuppressed mice [43], so it was inferred that an (egg) antigen or antigens induced those antibodies: However, no single antigen has yet been implicated in the phenomenon. Experiments with *S. mansoni* and *S. bovis*, involving transfer of immune cells and serum to infected deprived mice, showed that the rate of schistosome egg excretion of both parasite species correlated numerically with the diameter of egg granulomas in the livers of the recipient mice [47]. The process of extravasation of eggs and their passage through intestinal tissue to the gut lumen is undoubtedly complex, and appears also to involve blood platelets [48], the latter probably in the initial adherence of the egg to the endothelial surface.

As demonstrated first by [49], the *S. mansoni* egg granuloma is an immune (delayed hypersensitivity-type) response, but no single schistosome antigen has been identified as the causative agent: Rather, a complex of glycoconjugates may be responsible (van der Vijver et al., 2006). The hepatotoxicity that occurs in the absence of granuloma formation in immunosuppressed mice intuitively led to the conclusion that granulomas protected host tissue against toxic egg products [45,46]. Paradoxically, however, immunosuppressed mice with heavy *S. bovis* infections suffer no such hepatotoxicity and in fact survived longer than comparably-infected immunologically-intact controls [50]. Literature on the much-studied schistosome egg granuloma nevertheless still commonly implies that protection of host tissue is an important function of this cell-mediated immune response, though the results of an attempt to compare the relative hepatoprotective effects of transferred immune cells and immune serum in *S. mansoni*-infected deprived mice suggested the latter was more effective [51]. Promotion of schistosome egg excretion (and thus enabling continuation of the parasite's life-cycle) may therefore be the prime biological function of the schistosome egg granuloma [47] and immune-facilitated dispersion of infection may be a feature that this infectious disease has in common with tuberculosis [52].

A search for the *S. mansoni* egg antigen responsible for the hepatotoxicity reaction in infected, immunosuppressed mice was more successful. Small volumes of sera from *S. mansoni*-infected mice and mice immunized with *S. mansoni* eggs could protect the livers of infected, immunosuppressed recipients and examination of immunoprecipitating antibody patterns in the hepatoprotective sera indicated that *S. mansoni* egg antigen omega-1 was the likely hepatotoxin [53].

During the course of the experiments to identify the hepatotoxin cation-exchange chromatography was used to prepare six fractions from *S. mansoni* egg homogenate. Samples of each of the fractions were forwarded to Chris Draper and colleagues at what was then the Ross Institute of LSHTM to test for sensitivity and specificity in detection of human anti-schistosome antibodies in enzyme-linked immunosorbent assay (ELISA). The sixth cation exchange fraction (CEF-6) containing omega-1 and another antigen we named alpha-1 [53], was found to give the best serodiagnostic results in terms of sensitivity and specificity and intensity of reactivity with human *S. mansoni* infection sera [54]. The result was consistent with the observation that in western immunoblots such sera had antibodies which reacted with only omega-1, alpha-1 and a third antigen, kappa-5 (Hamilton et al., 1998).

In 1980-1981 the World Health Organization (WHO) organized an interlaboratory collaborative trial of antigens for immunodiagnosis of schistosomiasis. CEF-6 performed well as one of a panel of 21

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S. mansoni antigens and immunoassay combinations that the WHO trial tested: it gave 91.7% sensitivity and 90.0% specificity in ELISA, 'demonstrated a high degree of sensitivity and specificity' in an indium slide immunoassay, was the only preparation to give a significant positive correlation between the ELISA absorbance value and faecal egg count, and gave the highest rate of conversion towards sero-negativity after chemotherapy [55]. Despite its success in the WHO trial and demonstrations of potential usefulness in the field [56,57] and clinic [58], CEF-6 is not used in routine diagnosis of human schistosomiasis by antibody detection, perhaps because it is too costly compared with unfractionated *S. mansoni* soluble egg antigens (SmSEA) which are now in relatively common use in ELISA.

During the 1980s and 1990s our work on the two antigens in CEF-6 led to refined methods for their purification and furthered their characterization [59,60]. This was of course the period in which immunological research was transformed by the Th1/Th2 paradigm [61,62], the immunology of parasitic infection being also profoundly affected by this revolution.

Many studies have now shown that *S. mansoni* eggs confer a strong Th2 bias on immune responsiveness [63]. Early in 2002, however, I received a draft manuscript written by Gabi Schramm and Helmut Haas (Borstel Research Institute, Germany) describing an interleukin-4-inducing factor (named IPSE) that they had purified from *S. mansoni* eggs and which had the novel property of activating basophils to release the Th2 cytokine IL-4 via interaction with cell surface-IgE in a non-antigen-specific way. To my surprise I was listed as an author and on enquiring why, was informed that this was because I had supplied *S. mansoni* eggs from which mRNA had been extracted and the gene for IPSE cloned and expressed, a transaction that I had long forgotten about.

Results of polyacrylamide gel and western blot analyses of IPSE in this paper [64], were very reminiscent of the dimeric structure of alpha-1 [60], one of the two antigenic constituents of CEF-6 that we had identified in SmSEA some 20 years earlier [53]. A collaborative study confirmed that Borstel's IPSE was indeed the same as our alpha-1, hence the current name for this molecule: IPSE/alpha-1 [65]. The evidence that IPSE/alpha-1 has an inherent Th2-biasing effect was consolidated by demonstrating its activity *in vivo* (Schramm et al., 2007). IPSE/alpha-1 is a new member of the βγ-crystallin superfamily of proteins and is considered to activate basophils via its unique IgE-binding crystallin fold [66].

Omega-1, the hepatotoxic *S. mansoni* egg antigen in CEF-6, has also been cloned and expressed in recombinant form and has homology with the T2 family of ribonucleases [67]. This molecule also has a potent Th2-conditioning effect *in vitro* and *in vivo*, mediated through its activity on dendritic cells [68]. The gene for kappa-5, the third immunodominant antigen in SmSEA, has likewise been cloned and expressed [69], but its role in the *S. mansoni* host-parasite relationship is not known.

IPSE/alpha-1, omega-1 and kappa-5 are glycosylated and their respective glycanic constitutions have been analysed. The N-glycosylation sites of both IPSE/alpha-1 [70] and omega-1 [71], carry core difucosylated diantennary glycans with one or more Lewis X motifs in the antennae. The glycosylation of kappa-5 is distinct from that of the other two SmSEA molecules, as it comprises unique triantennary glycans composed of both difucosylated and xylosylated core regions and immunogenic GalNAc1–4GlcNAc (LDN) termini [72]. As mentioned above, glycoconjugate complexes may be responsible for granuloma induction (van der Vijver et al., 2006), but the extent to which the glycosylation of these three SmSEA molecules are involved in granuloma formation has not yet been studied.

Frank Cox writes about heterologous immunity

In the 1960s and 1970s I was working in the Zoology Department at King's College London where my lab was interested in immunity to blood parasites in rodents and where we maintained six different species of intraerythrocytic protozoa in mice and frozen aliquots. Four were malaria parasites as possible models for human malaria and two were piroplasms as possible models for the cattle disease, babesiosis.

We had already established, not unexpectedly, that infection with a particular species protected against the same species (homologous immunity) but also that in each of the pairs, (*Plasmodium yoelii* (Py)/*P. berghei* (Pb), *P. chabaudi* (Py)/*P. vinckei* (Pv) and *Babesia microti* (Bm)/*B. rodhaini* (Br)) mice that had recovered from infection with the avirulent species (the first in each pair) were protected to a greater or lesser extent against the virulent form (heterologous immunity) [73]. We then began to look for shared antigens and the possibility that antigens from a virulent parasites might be a basis of a vaccine to protect mice against virulent parasites. Our dream was to use this information for the development of a vaccine to protect humans against infection with the most dangerous malaria parasite, *Plasmodium falciparum*.

It was then that we discovered the most unexpected result: heterologous immunity between parasites belonging to different genera. It all began by accident. One Friday night it was my responsibility to test some frozen aliquots for viability. I had had a very busy teaching day so by the time I got to lab everyone else in the team had left leaving me with cages of mice that my technician had prepared and labelled. On the following Monday, we were surprised to discover that the mice injected with the virulent parasite, *P. vinckei,* showed no parasites but when we retested the same aliquot the mice all became infected. I was then told that the mice that had not become infected were not actually clean mice, as they should have been, but were mice that had recovered from the avirulent piroplasm, *B. microti*. Acting on a hunch, I set up a controlled experiment in which we infected mice that had recovered from *B. microti* with *P. vinckei* but none became infected. It then dawned on us that we had discovered that heterologous immunity between blood parasites was not confined to the genus *Plasmodium* but extended to a distantly related genus, *Babesia*, thus challenging the central tenet of immunology that immunity to one organism was specific to that organism. We carefully repeated these experiments again with the same results and sent off a paper to *Nature* which they published immediately [74].

One of the most influential parasite immunologists in the UK, Professor Sydney Cohen FRS, told us that our experiments had 'turned immunology on its head' and encouraged us to embark on a long series of cross-immunity experiments that extended our earlier investigations. Another person who was interested in our results was an Australian immunopathologist, Ian Clark, then working at the Royal College of Surgeons a few minutes' walk from King's College, who was interested in non-specific immunostimulation. Among the immunostimulants he was using was BCG. We then injected mice with BCG by various routes and challenged with them *P. vinckei*, *P. yoelii*, *B. rodhaini* or *B. microti* and found protection similar to that that we had described in the heterologous immunity experiments: another paper to *Nature* [75]. BCG is a living immunostimulant so we repeated the experiments with a killed organism, *Corynebacterium parvum,* with much the same results and extended our studies to other immunostimulants and began to think about the possibility of non-specific immunisation as a weapon against malaria [73].

We had now established that immunity to certain blood parasites could be induced by infection with a heterologous organism or by immunostimulants so we needed to understand the mechanisms involved. One clue came from the fact that Ian Clark, using light- and electron- microscopy, had observed that during the recovery phase of the *B. microti* infection parasites were seen to be dying within the red cells, observations that we repeated. We surmised that this was due to low molecule substances diffusing across the red cell membrane and Ian Clark suggested that these might be reactive oxygen intermediates (ROI), superoxides, peroxides and hydroxyl radicals, or reactive nitrogen intermediates (RNI). At this stage, we discovered that the generation of these toxic molecules actually did as much damage to the host as to the parasite. We then realised that we not going to get funding to further this line of research and dreams of a trip to Stockholm quickly evaporated.

Heterologous immunity is now known to be a more widespread phenomenon than we had expected and the rest of this chapter will concentrate on schistosomiasis. George Nelson (one of Leiper's successors as Professor at the London School of Hygiene and Tropical Medicine) and his colleagues found a considerable amount of heterologous immunity between *Schistosoma* spp. For example, in mice infected with *Schstosoma bovis* or *S. matthei* subsequent *S. mansoni* infections were reduced by 75%-85% but *S. bovis* did not protect

against *S. matthei* [76] and later that *S. haematobium* did not protect mice against *S. mansoni*. Over the next five years Nelson's team wrote a series of papers under the heading *Studies on Heterologous Immunity in Schistosomias*, well worth reading in full, that extended these studies to other species of *Schistosoma* and other hosts including monkeys and baboons.

Heterologous immunity between more distantly related organisms and schistosomes has received very little attention. One publication predates the experiments described above. In voles co-infected with *Schistosoma mansoni* and *Plasmodium berghei* the malaria infections were enhanced when both infections were given within two weeks of each other but depressed when the mice were infected with the malarial parasite seven weeks after infection with the schistosome [77]. Among the relatively few similar investigations, *Plasmodium chabaudi* infections in mice were enhanced in mice when the schistosome was given eight weeks earlier, *Trypanosoma cruzi* infections were more severe in mice infected with *S. mansoni* as were infections with *Toxoplasma gondii*, *Entamoeba muris*, *Trichomonas muris* and *Spironucleus muris* and in hamsters subsequently infected with *Leishmania infantum* [78], for more information and references. These experiments suggested that mice experienced a phase of immunodepression following infection with schistosomes, now a well-known phenomenon and that this inhibited the ability of the host to mount an effective immune response against subsequent infection with protozoa. The reciprocal is also the case: Infection with *Plasmodium yoelii* inhibited the development of schistosome granulomas in mice and also in the *T. gondii/S. mansoni* combination. As granuloma formation in both these cases results from enhanced immune responses the assumption must be that the protozoan infection depresses the immune response to subsequent infections with heterologous organisms [78,79].

This is not the place to discuss the mechanisms involved in heterologous immunity, nor to extend this discussion to include viral or bacterial infections, but the important point is that, in humans and other animals, exposure to unrelated infections can determine the outcome of subsequent schistosome infections. In the wild there cannot be many, if any, hosts that have not been, or have harboured another infection and that this is something that should be taken in to account in any field studies. This seems to be rich vein that needs to be mined and Leiper would probably have agreed.

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3 DISCUSSION, CONCLUSIONS AND FUTURE PERSPECTIVES

We can see from these accounts of personal experiences that there is a very great variety of conditions that motivate and excite the research worker and these relate to the personality of the scientist concerned. Leiper worked with a logical method and at times good relationships with colleagues, such as Atkinson, were put at risk. He valued painstaking observation and would always recommend "another closer look". Dogmas need to be challenged and Leiper did this in his approach to the ideas of Loos, who denied that there was an intermediate host in schistosomiasis. Wilson very carefully challenged the dogmas of concomitant immunity and good laboratory observations, such as those of his colleague Coulson that worms from monkeys were pallid, led to a breakthrough in studying the feeding habits of the schistosome. Vieira, Cox and Kusel describe that chance findings in the laboratory to have played a big part in their research. They also describe the crucial impact of having students working in the laboratory. Colley stresses that good colleagues with whom ideas can be readily discussed have played essential parts in the research. Cioli has a clear and logical approach to the mechanism of action of hycanthone and Livia Pica-Mattoccia has been an important colleague and friend. Coelho similarly has a well-designed project on snails resistant to miracidia of *S. mansoni* and friendships with colleagues contributed greatly to the success of the project which took decades to complete. Harnett describes the excitement of experiments using a newly developed assay. He also finds the inspiring university teaching of Adrian Hopkins to have changed his career path. Ruppel uses immunodiagnostic methods in very challenging field conditions in a Mali market-place, helped by inquisitive young boys! Ruppel also makes the important point that, at the time, the scientist works on the accepted methods and paradigms. It is only with hindsight that he/she finds that these paradigms have been misleading. However, the mind that constantly challenges is in danger of being unendingly critical. Doenhoff has a great ability to challenge accepted ideas and his imagination and persistance ends his account here with a possible explanation of the hygiene hypothesis. Colley describes the importance of guiding students carefully into research by getting their own results and allowing their own interpretations. Colley recognised the beauty of the eosinophil, Cioli felt awe and admiration of the parasite and Kusel saw wonder when observing the flame cell of the excretory system in the egg and the adult worm. All our scientists found this beauty in working with the living parasite. This feeling is essential for sustained work and progress in any project.

What other things have we learned from these discourses? Students have always been an important factor in the history of the discovery.

The stimulating effects of students in the laboratory has been described by [80] and current attempts to separate teaching from research in Universities is a flawed and superficial understanding of both. We have attempted to describe our experiences in scientific research

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much as indicated by [82-85]. The creative worker is an adventurer, an explorer and has a close relationship with the object being studied. There is a spiritual dimension to all the work, especially during discovery.

We will end our stories with advice to our grand-children: Love your subject and show love for others. Always travel with humility. If you have an idea, try it out immediately, form good friendships and never denigrate others, immerse yourself in a problem and feel free to discuss it with anyone, and follow your results. Never give up. When you make a discovery never be arrogant and have a wider vision of creative work. Never be persuaded that religious feelings are separate from those seen during scientific investigation. Both are founded on wonder and love.

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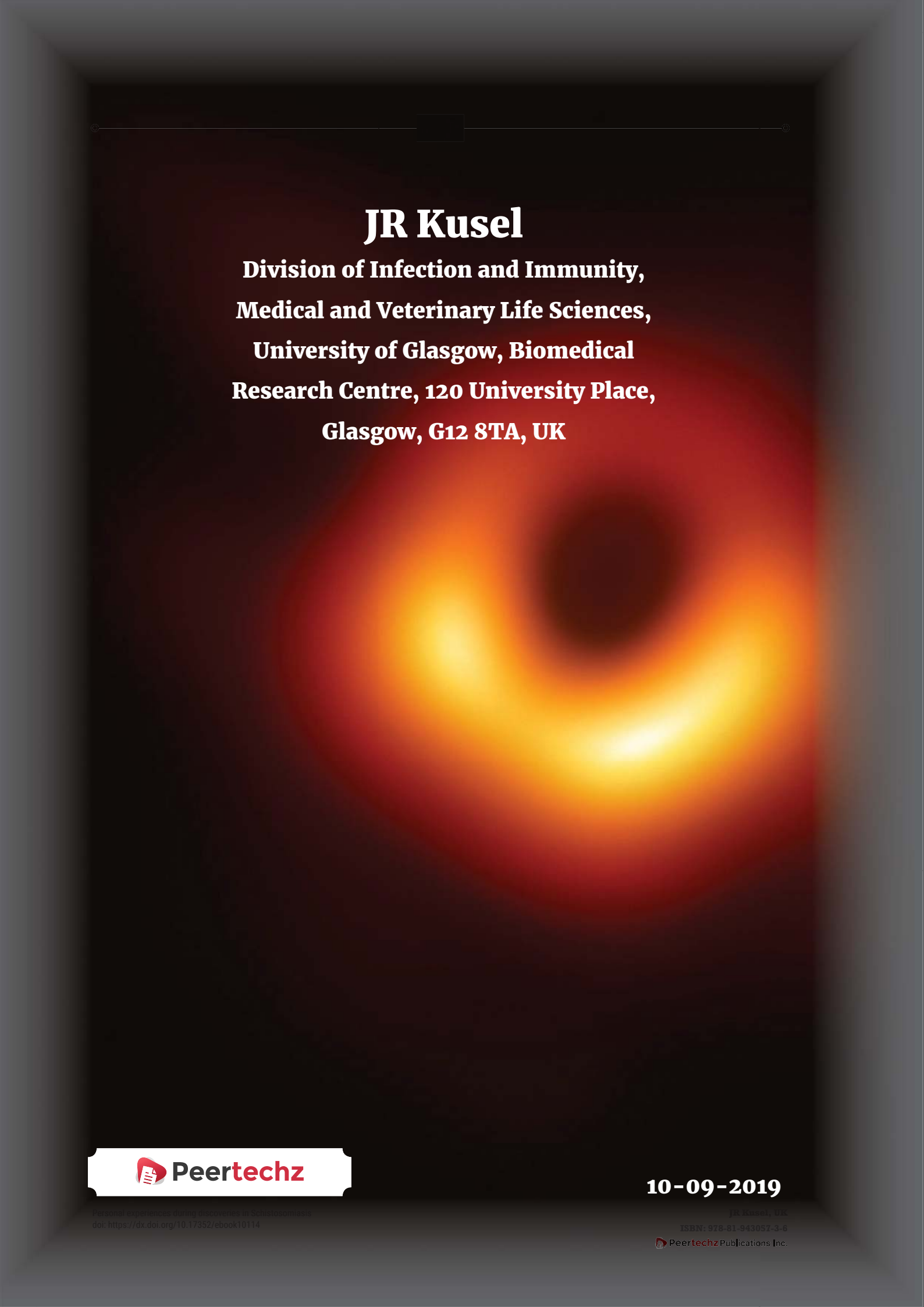
JR Kusel

Division of Infection and Immunity, Medical and Veterinary Life Sciences, University of Glasgow, Biomedical Research Centre, 120 University Place, Glasgow, G12 8TA, UK



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