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Ahead of the Curve

Hidden breakthroughs in the biosciences

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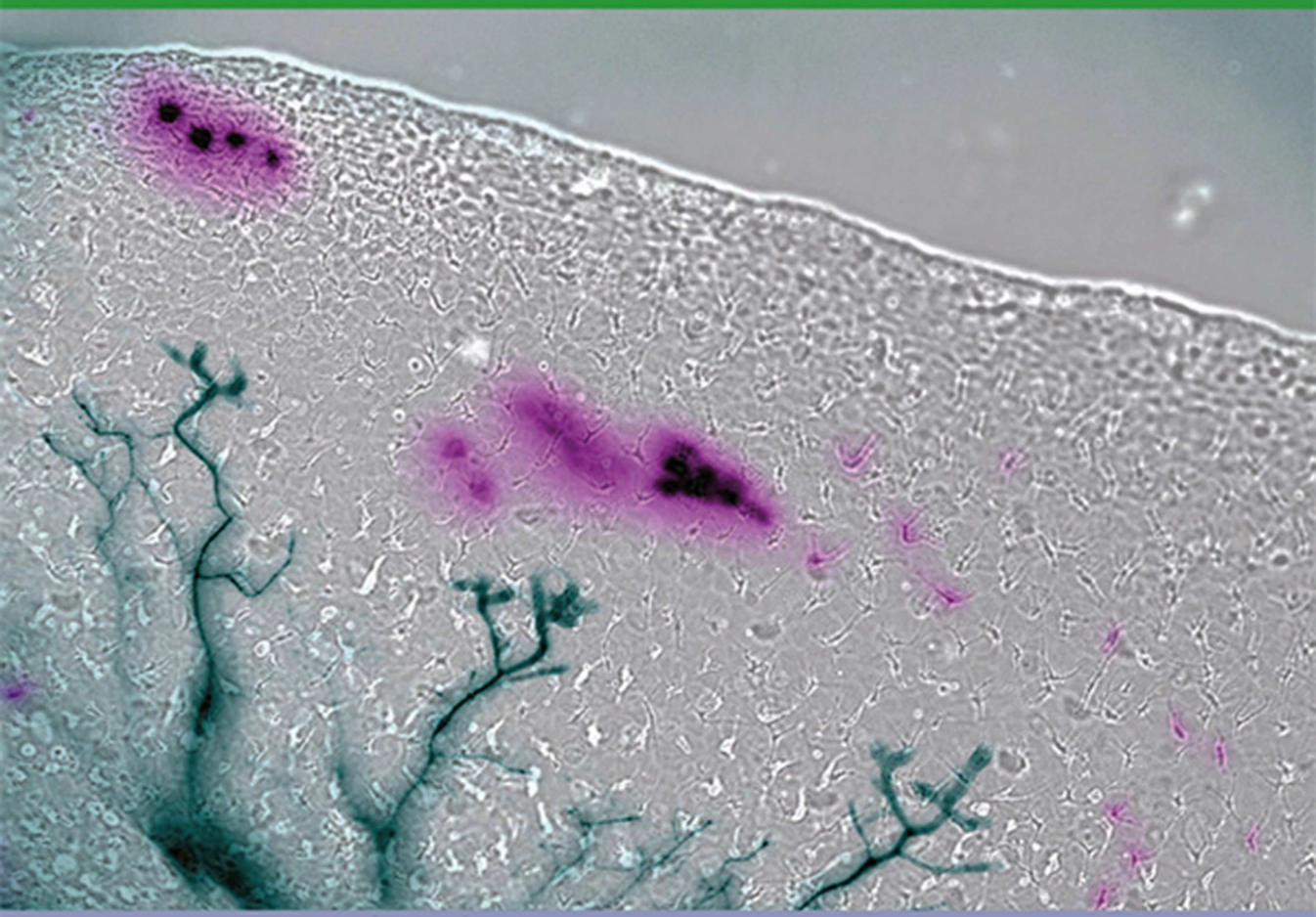

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This book is dedicated to all of the unsung pioneers—past, present, and future—who make fundamental discoveries 'Ahead of the Curve', and advance science by bearing the risks that such creativity entails.

Contents

Preface

The popular conception of science is of a continuous, steady, upward climb of progress. The reality is not as simple. Highly significant discoveries may often stay unrecognized for decades, if they conflict with the current paradigm or extend it in ways hard to imagine at the time. Recently, we were bemoaning how important scientific signals get lost in the noise generated by the sheer volume of data and the dominance of the latest trend, whatever it may be. Once we started listing our own favorite unknown classics, and soliciting other titles from colleagues, we realized that there are substantially more than enough such forgotten, never-noticed, or ignored papers to fill a book. Re-viewing of research that identifies exciting and influential new aspects of science (here we focus on biology), well in advance of mainstream thought, provides fascinating and instructive case studies for improving our ability to recognize influential findings that significantly revise our understanding of the world. They serve not only as studies of the history of science, but as windows on the dynamic process by which knowledge progresses; moreover, by examining case histories of good science derailed by something other than evidence, we may learn how to better interpret new ideas and data that contradict our expectations.

We decided to start with evidence that important things really do get missed, by including important things that were, in fact, missed. Part I therefore comprises papers describing theories and findings that are now widely accepted, but that received little or no attention when they were first published. Follow up confirmation took years to decades, and in the case of physician hygiene, over a century to happen. In some cases, the work itself was rediscovered, in others, someone else found the same thing. Part II is a compilation of papers that we believe describe important ideas and results that are, as yet, not widely known or used. We do not claim certainty that all of these papers will someday earn renown or prove useful; our argument is only that the ideas presented are supported by data and deserve closer inspection. Our claim is that all of these papers raise important questions or describe novel and essential ideas. Some go against conventional wisdom, some point out the importance of testing assumptions, still others contain known facts that are underused.

Michael Levin is eternally grateful to his parents, Benjamin and Luba, and his wife Kristin, for all of their support of his efforts to pull 'The Curve' in novel and unusual directions. This book is for Sam and Arthur, with the hopes that your work may someday be featured in a future volume of this series.

Dany Spencer Adams expresses immeasurable gratitude to the always supportive Adams–Olden–Silvan family, to wonderful friends, and to her husband Joe. This work is for Zachary, Ariana, Jeremy, Mia, Simone, Ariele, and Ben, with hope that their curiosity will lead them beyond what anybody else tells them is enough to know.

Acknowledgements

We are very grateful to Scott Gilbert, Ray Keller, Larry Stern, Richard Nuccitelli, Lev Beloussov, Wendy Brandts, Sara Walker, Jack Tuszynski, Susan Ernst, and Edward J Steele for suggestions of papers to include and for contributing their perspectives on the importance of the works. We are also grateful to numerous colleagues who suggested other papers and topics, which hopefully will be covered in subsequent volumes of this series. William F Baga did critical logistical work. We are also grateful for the guidance and expertise provided by IOP Publishing, especially Daniel Heatley, and Jessica Fricchione, as well as Chris Benson and Jacky Mucklow.

Author biographies

Michael Levin

Michael Levin was born in Moscow, Russia in 1969, and emigrated to the North Shore of Boston with his family in 1978. He worked as a software engineer interested in artificial intelligence prior to moving from computer science to biology. He received a PhD from Harvard Medical School in genetics, and did post-doctoral training working on the molecular mechanisms of embryogenesis. His first independent laboratory was at Forsyth Institute in 2000, establishing a novel research program in the biophysics of biological pattern control. The group moved to Tufts University in 2008, where he collaborates with computer scientists, bioengineers, and workers in cognitive neuroscience. His lab (www.drmichaellevin.org) now works on a number of frontier topics, including the communication and computation among non-neural cells that underlie control of biological growth and form, somatic memory and learning outside the brain, and artificial intelligence approaches to helping understand complex biological phenomena. He is currently Vannevar Bush Professor in the department of Biology, directing the Allen Discovery Center at Tufts University (allencenter.tufts.edu).

Dany Spencer Adams

Dany Spencer Adams is a Research Professor in the Department of Biology at Tufts University, a Principle Investigator in the Tufts Center for Regenerative and Developmental Biology, faculty in the EBICS program at MIT, and the author of *Lab Math: A Handbook of Measurements, Calculations, and Other Quantitative Skills for Use at the Bench*. Her movies of bioelectric signaling in developing Xenopus embryos have been seen on Discovery's Curiosity. She blogs about numbers at LabMath.org. She has always been interested in looking at questions that are off the beaten path, specifically in the area of biomechanics and biophysics during morphogenesis. As an undergraduate at UC Berkeley, she did research with Drs Ray Keller and M A R Koehl. She got her PhD from The University of Washington where she studied with Drs Thomas Daniel and Garret Odell. She started her independent career as an assistant professor in the Biology department of Smith College. After several years, she decided to focus on research full-time, starting what would turn out to be a long-term collaboration with Dr Michael Levin at The Forsyth Institute. There she began her studies of craniofacial development, specifically the roles of ion-flux dependent phenomena during differentiation and morphogenesis of cranial neural crest and placode-derived structures. Her current work also touches on ion flux during regeneration and during transformation, with a technical emphasis on adapting ion and membrane voltage imaging techniques for use in vivo in embryos. Because of the caliber and nature of their collaborations, Dr Adams joined Dr Levin for the move to Tufts University in 2008.

Introduction

We created this book for two purposes. First, to highlight some specific topics in biology that we consider especially fascinating; this includes the roles of biophysical forces, approaches to mathematical understanding of living systems, non-genic inheritance, and the relationship between memory and the body. Our second purpose is to provide papers that have instructive lessons for us in the present. Most of our entries are accompanied by a Perspective, written by a current expert in each sub-field, who provides a personal commentary on the significance of the work and why it was not recognized as the advance that it was. Taken as a collection, these stories have lessons for us about why key findings get missed and most importantly, how to spot important breakthroughs in the future to reduce the time before their positive impact is felt. Many of the key studies (even ones that were immediately recognized as major advances) are published in journals that are not considered the "top tier". Especially today, journals are highly stratified, and editors exert a significant filtering function over submissions to try to publish only "high impact" papers; guessing this in advance is a most difficult task and our intuitions can be improved by considering where the process failed to identify gems in the past.

We have endeavored to include papers from a variety of Biological disciplines, although our own interests and knowledge have biased the collection towards Cell and Developmental Biology. With most of the papers, we have included timelines or graphics illustrating the state of the field, i.e. the curve. These trend curves were made using PubMed's Results by Year Tool (Canese K. PubMed Discovery Tools. NLM Tech Bull. 2012 May-Jun;(386):e7) or MLTrends (http://mltrends.ogic.ca; Palidwor et al. (2010) J. Biomed. Discov. Collab. 5, 1-6;). As with any search, the choice of keywords determines what you find. In cases where keywords were not obvious, we had to use our own judgment; the terms used are supplied. We have also indicated, with an arrowhead, the date of the case study, to illustrate how far 'ahead of the curve' each paper is. It is important to remember, however, that definitions evolve, new jargon appears, there is no way to search directly for the influence of an idea, and counting instances does not tell you whether the terms were referred to positively or negatively. In other words, the graphs illustrate something both specific and vague, and should not be over-interpreted.

It is our hope that this compilation will inspire and educate by virtue of the value of the work collected, and that our contributions, of choosing and annotating papers, will facilitate those processes. We apologize to the many other researchers whose significant discoveries could not be covered here. While we can take neither credit nor blame for the science herein, we take full responsibility for our judgment and any errors in interpreting, contextualizing, or encapsulating. We have tried to uncover true breakthroughs, in the sense that an article contains the very first recognition of a topic, but this has not always been possible. Reports of earlier works have been followed up to the best of our abilities, but we have been constrained by factors including issues of translation, copyright, and availability of certain works.

Thus, unfortunately, we may have missed the truly deserving first author of an important idea; the irony of that has not escaped us. If that is found to be the case, we would like to know, and we agree that the fault lies with the other editor.

Michael Levin and Dany Spencer Adams
Medford, MA
November, 2016

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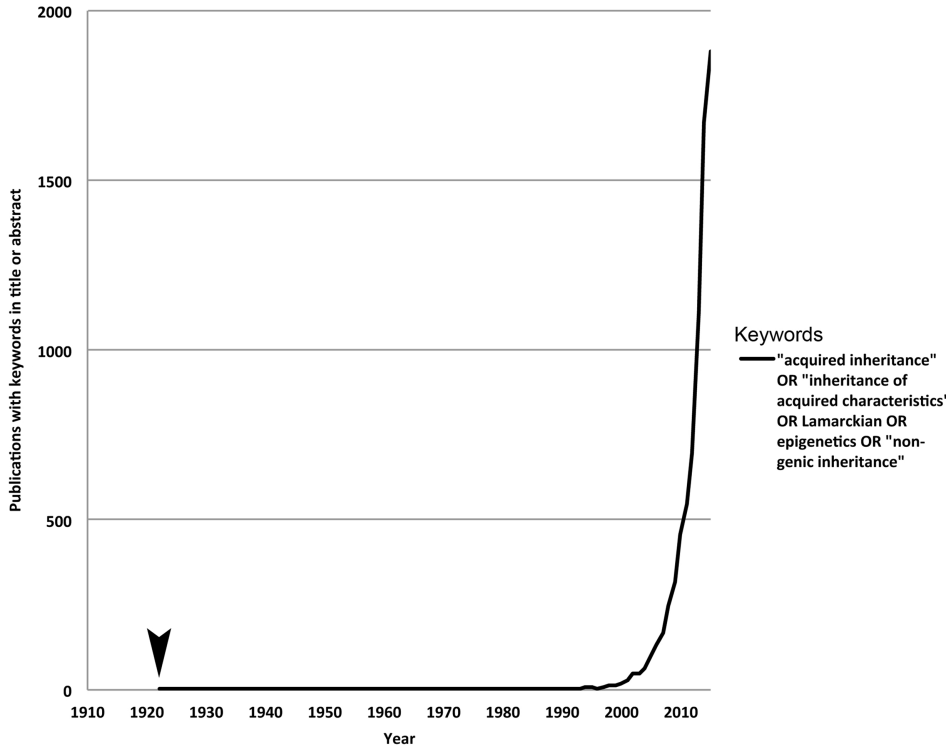
Chapter 3

Inheritance

Case Study 7: Induced Eye-Defects Can Be Passed On to Future Generations



Guyer (1922) The production and transmission of certain eye defects.

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Perspective on *The Production and Transmission of Certain Eye Defects*

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Origin of Congenital Defects: Stable inheritance through the male line via maternal antibodies specific for eye lens antigens inducing autoimmune eye defects in developing rabbits *in utero*

In April 1922 Michael F Guyer, Professor of Zoology at the University of Wisconsin, stood and addressed the International Congress of Othalmology in Washington D.C. He presented the results of a remarkable series of 'acquired inheritance' experiments which he and Elizabeth A Smith had been conducting at the Zoological Laboratory since 1916 (Guyer 1922). He also had with him live rabbits bearing defective eyes so that the gathered opthalmologists could examine some of the evidence for themselves. Guyer and Smith showed that specific antibodies for eye lens antigens crossed the placenta in rabbits producing offspring with severe eye defects. By itself this is perhaps not surprising. They then showed that these induced eye defects were passed on to future generations arising spontaneously in progeny rabbits via the male or female lines without any further immunization or antiserum treatment of subsequent parental rabbits with eye lens antibodies (Guyer and Smith 1918, 1920, 1924). In my view these transgenerational genetic data are on a historical par with Mendel's landmark publication on inheritance of genetic factors affecting characteristics of pea plants 60 years earlier.

I first wrote on this topic almost 40 years ago (Steele 1979 p. 59–63) yet the title of this perspective today has a contemporary ring about it—or at least I would like to think so. In fact I would like to think that in a few years it will be the type of title appearing on *many* biomedical and developmental biology papers particularly in relation to the origins of numerous congenital diseases afflicting mankind not just induced-eye defects. Thus Rachel Caspi (2010) of the National Eye Institute opened her recent review thus: 'Autoimmune and inflammatory uveitis are a group of potentially blinding intraocular inflammatory diseases that arise without a known infectious trigger and are often associated with immunological responses to unique retinal proteins' (also see Perez and Caspi 2015).

However there are other settings where the approach to 'acquired inheritance' we will discuss here are likely to be relevant viz. the origins of multiple sclerosis and its potential genetic transmission as a risk factor (van den Elsen 2014, Zager *et al* 2015) through the widespread use of 'EAE animal models', which use the Guyer and Smith immunization strategy to create experimental MS disease (experimental autoimmune encephalitis, EAE; Dendrou *et al* 2015).

The tissues of certain key vertebrate organs such as the eye, central nervous system, and the gonads are relatively immunologically privileged. Whilst the self antigens in these tissues are immunogenic in the host they are not normally exposed

to the adult immune system because of various blood–organ barriers (Blood–Brain Barrier; Blood–Retinal Barrier). Yet following injury to the tissue (disease, physical lesion) immune cells may gain access (antigen presenting cells and lymphocytes such as antibody producing B cells, and cytotoxic and helper/regulatory T cells). Quite vigorous immune responses can then ensue often becoming chronic as a waxing and waning autoimmune condition of increasing destructive severity in the individual and patient takes hold (indicative of an ongoing tugger war between the host tissue and its immune system with the latter usually winning). These diseases can be debilitating unless brought under control. It is this type of autoimmune disease which is our topic under discussion here described in the accompanying classic paper(s) published by MF Guyer and EA Smith between 1918 and 1924.

Thus autoimmunity affecting the eyes and central nervous system has to first breach the blood–retinal and blood–brain barriers. However there is another 'barrier' of equal importance breached in the Guyer–Smith experiments. This barrier is still surprisingly less well known to modern geneticists and biomedical scientists, because its existence is tacit and ingrained in our education system.

This is the Weismann Barrier, the tissue barrier protecting the germ cells from any type of somatic genetic influence from the body's somatic cell populations of circulating white cells in blood and lymph, as well as the cells in the fixed tissues and organs. Thus Weismann's Barrier traditionally *forbids* the flow of genetic information (DNA/RNA) from somatic cells to germ cells (whereupon it could be integrated into germline DNA thus affecting a possible 'directional' genetic change in the offspring, Lindley 2010). Accordingly the Weismann Barrier forbids the inheritance of somatically acquired characteristics, such as somatically hypermutated (Steele 2016) antibody V genes (Steele and Lloyd 2015)—it forbids what is known as 'Lamarckian modes of inheritance'. The reader can Google the significance of this barrier to theories of biological evolution—but the Weismann Barrier is *the* central pillar of mainstream genetics particularly the neo-Darwinian New Synthesis fashioned in the 1930s and 1940s giving rise to modern Population Genetics (Steele 1979, Steele *et al* 1984, Steele *et al* 1998, Steele *et al* 2002, Lindley 2010, Steele and Lloyd 2015).

Discussing or researching this topic openly has been a highly hazardous exercise for one's academic and scientific career. Therefore *many* scientists *do not entertain doing such research.* The present author nevertheless crossed that red line over 40 years ago (Steele 1979, Gorczynski and Steele 1980, 1981, Steele *et al* 1984, Steele *et al* 1998, Blanden *et al* 1998, Steele *et al* 2002, Lindley 2010, Steele and Lloyd 2015). In my view this area of genetics is of the utmost importance to explore, rationalise and explain, *particularly now* in this era of whole genome sequencing and personalised genomic medicine.

The heat and intensity of this battle has diminished some what in the past 20 years – but not the conviction among mainstream geneticists that the 'acquired inheritance' proposition is both wrong if not absurd and a contradiction in terms. Indeed Max Planck's observation at the turn of the 20th Century that one needs to wait for the vanguard of 'dogmatic orthodoxy' to literally die out certainly seems to apply in this case. No doubt the publication of numerous papers describing

controlled and induced transgenerational phenomena particularly via the male has forced mainstream science to take the ideas behind such work far more seriously today (Campbell and Perkins 1988, Lindley 2010).

Thus in recent years there has been a flowering of reports on so called Lamarckian 'Acquired Inheritance' or 'Epigenetic' phenomena in many animal and plant systems where the claim is clearly made either overtly or tacitly, that Weismann' Barrier has been breached and is clearly permeable—but only up to a point (Jablonka and Lamb 1995, Rassoulzadegan *et al* 2006, Whitelaw and Whitelaw 2008, Grossniklaus *et al* 2013, Sharma 2013, Devanapally *et al* 2015, Sharma *et al* 2016). If the fine print is read carefully you will find the disclaimer: 'Epigenetics, the transgenerational transfer of phenotypic characters without modification of gene sequence' (e.g. Ho and Buggren 2010). So the inheritance is 'soft' and thus 'regulatory' and reversible in nature, involving DNA methylation-type effects and miRNA inhibitory effects on protein synthesis (translation), quantitatively controlling the magnitude and intensity of the 'transmitted' phenotype in the progeny.

The present author has spent 40 years studying the conditions for the transmission of 'hard' Lamarckian inheritance effects in the immune system of mammals (mice, rabbits, humans) whereby the outcome *must be* the integration of the selected somatic gene mutation into the germline DNA (Steele 1979, Gorczynski and Steele 1980, 1981, Steele *et al* 1984, Steele *et al* 1998, Blanden *et al* 1998, Steele *et al* 2002, Steele and Lloyd 2015). So there is still a type of stand-off here—although in recent tumor systems in mice by the Spadafora group (Cossetti *et al* 2014) and in microscopic worms (*C. elegans*, Devanapally *et al* 2015) forms of clear cut soma-to-germline transmission have been demonstrated. And 10 years ago the very clear demonstration in mice that somatic regulatory microRNAs (miRNA) can been shown to be transmitted in a non-Mendelian manner to progeny via spermatozoa (Rassoulzadegan *et al* 2006).

The problem is often the long term nature as well as the level of technical and logistical difficulty in conducting a soma-to-germline experiment in the current prejudicial research funding environment. Further, in the case of the immune system the key genes, the variable region genes (V) involved in foreign antigen recognition by B and T cells exist not as single genes but as *highly similar* genes in repertoire clusters spanning at least a megabase in the germline (where $N = 50 - 150$ V elements). These loci are very difficult to analyse, particularly in the case of human antibody V clusters. It is therefore a challenge to accurately and easily DNA sequence on the scale required for animal experiments or inheritance studies on human subjects (Steele and Lloyd 2015). Strategies based on informative three-generation pedigrees in humans have been proposed and are likely to be taken seriously for the heavy chain IGHV gene cluster on human chromosome 14 at band 14q32.33, but it will still require significant technical innovation in long-read DNA sequencing (Steele and Lloyd 2015).

Let us now summarise in more detail the results obtained in the experiments of Guyer and Smith. From a perspective of 100 years these are remarkable and revolutionary experiments. In my opinion these data provide the *first clear-cut*

controlled studies unequivocally showing the germline transmission of a specific antibody-induced somatic character thus demonstrating the inheritance of an acquired autoimmune disease (the reader is advised to ignore the negative comments in Wiki and read Guyer–Smith very carefully and then consult Steele 1979 p. 59–63 and references therein, and this paper).

Here is a dot-point summary of some of their key findings, but the reader is urged to carefully read all the papers, particularly the final 1924 paper:

- In the first experimental line of rabbits bearing transmitted induced eye-defects specific antiserum to eye-lens and associated proteins was raised in fowls after grinding an emulsion of rabbit eye-lens tissue into normal saline. This antiserum was injected in multiple doses to pregnant does during gestation. The lethal toxicity of this manoeuvre on both the embryo (spontaneous abortions) and the occasional death of a mother was apparent—as would be expected. However from about 15 pregnant mothers treated with anti-eye lens antiserum 62 offspring were produced and 11 had clear eye defects. These defects were propagated through to the ninth generation either via the male and/or female line.
- The control serum and other vaccination exposures are very significant, as such mothers produced progeny with *no eye defects*:
  * Normal fowl serum or fowl anti-rabbit testis (50 progeny with no eye defects)
  * A large control group of progeny arising from immunised rabbit ($N >$ 500) where mothers (and males in some cases) were hyperimmunised with different types of antigens. It is informative to quote Guyer and Smith directly at this juncture:

> *'…we, together with others in our laboratory, have been doing many experiments in which shortly before or during pregnancy female rabbits have been injected with typhoid fever vaccines or living typhoid germs, or with various kinds of foreign serum or serum immunized against proteins other than lens, and in not a single one of more than five hundred young born after such treatments has there been a case of eye defect. But even should the defect have originally been a general rather than a specific one, it is obvious that the germinal condition sooner or later must have become specific since the anomaly reappears generation after generation without any recognizable accompanying malformations of other parts of the body.' (Guyer and Smith 1924)*

These results clearly show that the effects induced were specific for eye lens and associated eye proteins, and do not normally appear in rabbit populations at the frequency observed by Guyer and Smith in their test experiments.

- The Transmission of the defects by male progeny with defective eyes rules out ongoing direct maternal antibody effects down many (up to 9) generations of breeding and thus suggests stable genetic inheritance associated presumably (in modern parlance) with chromosomal DNA sequences.

- Backcrossing experiments to show that the transmitted defects, which may disappear in one or two generations, can reappear again suggesting a type of Mendelian recessive inheritance. Although there are clearly several specific genetic factors involved as they point out.
- Establishing a separate line via the defective-eyed male progeny where they directly induced the production of anti-eye lens antibodies in the pregnant mothers by multiple vaccine doses of rabbit eye lens emulsion in normal saline. This demonstration is very important and remarkably modern.

The current animal models for induced autoimmune disease for Uveitis (EAU) and Multiple Sclerosis (EAE) depends *precisely* on this type of autoimmunization strategy first used by Guyer and Smith (Caspi 2010, Perez and Caspi 2015, Dendrou *et al* 2015). The modern twist would be for the experimenter to set up EAU or EAE disease in pregnant mothers (or males for that matter cf Gorczynski and Steele 1980, 1981, Steele 1984a, 1984b, Steele *et al* 1984) and then breed them to see if tissue-specific defects appear in progeny—with or without possible co-factor manoeuvres which might involve mild or graded injury to target tissues in progeny (to allow easier lymphoid and white blood cell access to targets and speed up the assay read-out).

An important rider I would add, after much experience of animal (mouse) breeding programs (Steele 1984a, 1984b, Steele *et al* 1984, 1986) is this: sufficient breeding pairs should be set up because not every autoimmune or antigen-treated mother (or father) will produce affected progeny (cf Gorczynski and Steele 1980, Figure 1)—Guyer and Smith also noted this. But the frequency is still high—a rule of thumb is that the expectation should be about 10% of treated breeding pairs will produce defective progeny at high frequency—and that upwards of 10% of progeny are likely to be affected (in the case of Guyer and Smith up to 15%–20% progeny were overtly affected).

The assay methods of 100 years ago were visual inspection backed by the standard ophthalmology techniques of the day. Note that ophthalmology experts examining the Guyer–Smith rabbits found *many more* eye defects than visually recorded so the eye-defect incidence in offspring is far higher than the 15%–20% reported. Modern Ophthalmology molecular assay and physical technical methods and whole genome DNA sequencing can be used today.

In wrapping up my interpretation of the Guyer–Smith data which I published in 1979 I stated the following:

> *'All these features are consistent with the following model (a) abnormal development of a target organ previously inaccessible to the immune system, (b) some of the "new" genetic information specifying this abnormal development pattern is transferred to and laid down in the offspring's germline, (c) this information is passed onto the next generation. Paralleling this now genetically specified organ defect is a chronic autoimmune response causing further damage to the target organ, and therefore favouring the germline fixation of high binding affinity, auto-specific V-region genes in the germline.' (Steele 1979 p. 62).*

I cannot improve on this summary. So I urge the interested reader, and budding young scientist, to read Guyer–Smith very carefully. Their classic papers still inform and speak directly to us 100 years later in easily understood scientific prose—and they are an instructive outline of careful objective scientific method.

Finally, there are now published results of ongoing current experiments and clinical observations on the etiology of human children suffering from Austism Spectrum Disorders (ASD) which are now even more compelling in the light of the Guyer–Smith experiments. These contemporary findings force all of us take the issues discovered by Guyer and Smith *very seriously* (Martin *et al* 2008, Bauman *et al* 2013; the whole field reviewed in Estes and McAllister 2015). In short these data, and their careful behavioural observations, make the compelling claim that maternal antibodies (IgG) for foetal brain antigens cross the placenta and affect long term behavioural damage causing the autism ASD syndrome in their children. These findings are so important we can quote the summary of the key findings from the group of David G Amaral (at the University of California, Davis) in their ground breaking 2008 paper using rhesus monkeys as the experimental vehicle:

> *'…. four rhesus monkeys were exposed prenatally to human IgG collected from mothers of multiple children diagnosed with ASD. Four control rhesus monkeys were exposed to human IgG collected from mothers of multiple typically developing children. Five additional monkeys were untreated controls. Monkeys were observed in a variety of behavioral paradigms involving unique social situations. Behaviors were scored by trained observers and overall activity was monitored with actimeters. Rhesus monkeys gestationally exposed to IgG class antibodies from mothers of children with ASD consistently demonstrated increased whole-body stereotypies across multiple testing paradigms. These monkeys were also hyperactive compared to controls. Treatment with IgG purified from mothers of typically developing children did not induce stereotypical or hyperactive behaviors. These findings support the potential for an autoimmune etiology in a subgroup of patients with neurodevelopmental disorders.' (Martin et al 2008).*

Thus Step I of the Guyer–Smith experiment has already been achieved in human subjects. When these ASD children grow up and produce their own offspring, and so forth down the generations, we will have secured Step II.

References

Bauman M D *et al* 2013 Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey *Transl. Psychiatry* **3** e278

Blanden R V, Rothenfluh H S, Zylstra P, Weiller G F and Steele E J 1998 The signature of somatic hypermutation appears to be written into the germline IgV segment repertoire *Immunol. Rev.* **162** 117–32

Campbell J H and Perkins P 1988 Transgenerational effects of drug and hormonal treatments in mammals: a review of observations and ideas *Progress in Brain Research* **Vol 73**; Boer G J, Feenstra M G P, Mirmiran M, Swaab D F and van Haaren F pp 535–53

Caspi R R 2010 A look at autoimmunity and inflammation in the eye *J Clin. Invest.* **120** 3073–83
Cossetti C *et al* 2014 Soma-to-germline transmission of RNA in mice xenografted with human tumour cells: possible transport by exosomes *PLoS ONE* **9** e101629
Dendrou C A, Fugger L and Friese M A 2015 Immunopathology of multiple sclerosis *Nat. Rev. Immunol.* **15** 545–58
Devanapally S, Ravikuma S and Jose A M 2015 Double-stranded RNA made in *C. elegans* neurons can enter the germline and cause transgenerational gene silencing *Proc. Natl Acad. Sci. USA* **112** 2133–8
Estes M L and McAllister A K 2015 Immune mediators in the brain and peripheral tissues in autism spectrum disorder *Nat. Rev. Neurosci.* **16** 469–86
van den Elsen P J, van Eggermond M C J A, Puentes F, van der Valk P, Baker D and Sandra Amor S 2014 The epigenetics of multiple sclerosis and other related disorders *Multiple Sclerosis and Related Disorders* 2014 **3** 163–75
Gorczynski R M and Steele E J 1980 Inheritance of acquired immunologic tolerance to foreign histocompatibility antigens in mice *Proc. Natl. Acad. Sci. USA* **77** 2871–5
Gorczynski R M and Steele E J 1981 Simultaneous yet independent inheritance of somatically acquired tolerance to two distinct H-2 antigenic haplotype determinants in mice *Nature* **289** 678–81
Grossniklaus U, Kelly W G, Ferguson-Smith A C, Pembrey M and Lindquist S 2013 Transgenerational epigenetic inheritance: how important is it? *Nat. Rev. Genet.* **14** 228–35
Guyer M F 1922 *The production and transmission of certain eye defects* (Washington D.C: Transactions of an International Congress of Ophthalmology) April 25–28, 1922
Guyer M F and Smith E A 1918 Studies on cytolysins I Some prenatal effects of lens antibodies *J. Expt. Zool.* **26** 65–82
Guyer M F and Smith E A 1920 Studies on cytolysins II Transmission of induced eye-defects *J. Expt. Zool.* **31** 171–216
Guyer M F and Smith E A 1924 Further studies on inheritance of eye defects induced in rabbits *J. Expt. Zool* **38** 449–75
Ho D H and Burggren W W 2010 Epigenetics and transgenerational transfer: a physiological perspective *J. Exp. Biol* **213** 3–16
Jablonka E and Lamb M J 1995 *Epigenetic Inheritance and Evolution:The Lamarckian Dimension* (Oxford: Oxford University Press)
Lindley R 2010 *The Soma: How Our Genes Really Work and Why That Changes Everything!* ISBN1451525648, POD book Amazon.com CYO Foundation)
Martin L A *et al* 2008 Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism *Brain Behav. Immun.* **22** 806–16
Perez V L and Caspi R R 2015 Immune mechanisms in inflammatory and degenerative eye disease *Trends Immunol.* **36** 354–63
Rassoulzadegan M, Grandiean V, Gounon P, Vincent S, Gillot I and Cuzin F 2006 RNA-mediated non-Mendelian inheritance of an epigenetic change in the mouse *Nature* **441** 469–74
Sharma A 2013 Transgenerational epigenetic inheritance: focus on soma to germline information transfer *Prog. Biophys. Mol. Biol.* **113** 439–46
Sharma U *et al* 2016 Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals *Science* **351** 391–6
Steele E J 1979 *Somatic Selection and Adaptive Evolution: On the Inheritance of Acquired Characters* 1st Edn. (Toronto: Williams-Wallace) 1979; 2nd Edn. Chicago, IL: University of Chicago Press. 1981

Steele E J 1984a Acquired paternal influence in mice. Improved reproductive performance of CBA/H males immunized with rat blood cells. *Aust. J. Exp. Biol. Med. Sci* **62** 155–66 (now Immunology and Cell Biology)

Steele E J 1984b Acquired paternal influence in mice. Altered serum antibody response in the progeny population of immunized CBA/H males *Aust. J. Exp. Biol. Med. Sci.* **62** 253–68

Steele E J 1986 Idiotypes, allotypes and a paradox of inheritance *Paradoxes in Immunology* ed G W Hoffman, J G Levy and G T Nepom (CRC Press Inc) pp 243–52

Steele E J 2016 Somatic hypermutation in immunity and cancer: Critical analysis of strand-biased and codon-context mutation signatures *DNA Repair* **45** 1–24

Steele E J, Hapel A J and Blanden R V 2002 How can DNA patterns of somatically acquired immunity be imprinted on the germline of immunoglobulin variable (V) genes? *IUBMB Life* **54** 305–7

Steele E J, Gorczynski R M and Pollard J W 1984 The somatic selection of acquired characters *Evolutionary Theory: Paths into the Future* ed J W Pollard (London: John Wiley) 217–37

Steele E J, Lindley R A and Blanden R V 1998 *Lamarck's Signature: How Retrogenes are Changing Darwin's Natural Selection Paradigm* (Reading, MT: Addison-Wesley-Longman)

Steele E J and Lloyd S S 2015 Soma-to-germline feedback is implied by the extreme polymorphism at IGHV relative to MHC *Bioessays* **37** 557–69

Whitelaw N C and Whitelaw E 2008 Transgenerational epigenetic inheritance in health and disease *Curr. Opin. Genet. Dev.* **18** 273–9

Zager A, Peron J P, Mennecier G, Rodrigues S C, Aloia T P and Palermo-Neto J 2015 Maternal immune activation in late gestation increases neuroinflammation and aggravates experimental autoimmune encephalomyelitis in the offspring *Brain, Behavior, and Immunity* **43** 159–71

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THE PRODUCTION AND TRANSMISSION OF CERTAIN EYE DEFECTS[1]

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By way of introduction to the discussion of eye defects, I wish to review briefly some points in the embryologic development of the eye. Although it will prove to be an old story to ophthalmologists, I feel, nevertheless, that by so doing I can get before you most effectively the materials I have to present.

Cleavage of the fertilized ovum, formation of the three fundamental germinal layers, and general embryogeny in the rabbit do not differ in any important ways from these same processes in other mammalian forms. Through the successive divisions which begin shortly after penetration of the ovum by the spermatozoon, a mulberry-like mass of cells enclosed by the *zona radiata* is built up. Some of the cells divide more rapidly than others, so that the resulting spherical mass comes to consist of a central group of larger, more granular cells surrounded by a superficial layer of smaller, clearer elements. Soon fluid appears between the central cells and the peripheral layer except at one side. As the liquid accumulates the entire mass becomes transformed into a fluid-filled vesicle consisting of a single layer of small transparent cells with the original central mass projecting from one side into the cavity. The outer layer, termed the *trophoblast,* is concerned only with the establishment of relations between the developing organism and the uterine mucosa. The inner mass is the part out of which the embryo is formed. At this stage the developing ovum is commonly termed the *blastodermic vesicle* or *blastocyst.*

Seen from without, the *germinal area* appears as a circular disc at the upper pole of the blastocyst. Within this disc the cells are rapidly shaping up into the two primitive germinal layers—*ectoderm,* and *entoderm.* By unequal growth the disc soon becomes oval, then more or less pear-shaped. At its smaller end a median denser streak, formed by a keel-like thickening of the ectoderm, appears. This is serologist has put important tools and ideas into the possession of the experimental biologist which may be utilized in new attacks upon certain fundamental biological problems.

The hemolysins, for example, discovered by Bordet in 1895, are now known to be special members of a general class of substances termed cytotoxins or cytolysins. For just as alien red blood-cells lead to the production of specific hemolysins, so various other materials, as leukocytes, nervous tissues, spermatozoa and crystalline lens—any foreign protein, in fact—when injected into the blood-stream of an unrelated species, will cause the formation of lytic substances more or less specific for the antigen used in the immunizing process. All cytolytic sera so far studied have been found to be more or less hemolytic, and it is probable that none acts exclusively

[1] Illustrated by lantern-slides and living animals.

upon its own antigen. While a particular cytolytic serum may affect some other tissues, it attacks the special tissue used as antigen much more vigorously.

Although presumably distinct from one another, the various classes of the so-called *antibodies*—precipitins, agglutinins, bacteriolysins, cytolysins or cytotoxins, etc.—seem to have many points of similarity, as, for instance, their method of origin, their reaction to heat, and, in some cases, their mode of operation. Chemically their natures are still unknown. Considerable evidence of their close association in some way with the euglobulin constituent of the blood is appearing in various recent researches.

To the biologist viewing this fascinating field, many questions arise. If, for example, it is possible to originate in living organisms antibodies which will destroy particular tissue-elements, is it not possible to secure similar selective action on certain parts of the developing embryo? May not serologic methods enable us to make a new attack upon the long-standing problem of the inheritance of somatic modifications, or that of provoking specific modifications in the germ through direct operation of external agents? If a special serum can be developed which will single out and destroy a certain element of the adult, is it not possible that there is sufficient constitutional identity between the mature substance of such a part and one or the other of its material antecedents in the germ, that the latter may also be influenced specifically by the serum in question? If external influences *can* be transmitted to the germ-cell, it is clear that in higher animals the one obvious means of conveyance is the blood.

In an attempt to find answers to certain questions of this kind I and my research associate, Dr. E. A. Smith, began various experiments some six years ago which we are still continuing. Among other things we undertook, by means of cytolysins, to produce antenatal effects in fetuses. Our main work in this direction has been on rabbits with fowl-serum immunized against rabbit-lens, although we have also experimented somewhat with mice and with guinea-pigs. I shall confine my discussion largely to certain eye-abnormalities we secured in fetal rabbits, and to the inheritance of such defects.

In our first experiments[2] the lenses of newly killed young rabbits were pulped thoroughly in a mortar and diluted with normal salt solution. About four cubic centimeters of this emulsion was then injected intravenously or intraperitoneally into each of several fowls. Four or five weekly treatments with such lens-emulsions were given. A week or ten days after the last injection the blood-serum of the fowls was ready for use. The rabbits had been so bred as to have their young advanced to about the tenth day of pregnancy, since from the tenth to the thirteenth day seems to be a particularly important period in the development of the lens. As we saw in reviewing the embryology of the eye, the lens is then growing rapidly and is surrounded by a rich vascular network that later disappears. From four to seven cubic centimeters of the immunized fowl-serum were injected intravenously into the pregnant rabbits at intervals of two or three days for from ten days to two weeks.

[2] Guyer and Smith, 1918, 1920.

A number of the rabbits died from the treatment and many young were killed in utero. Of sixty-one surviving young from mothers thus treated, four had one or both eyes conspicuously defective and five others had eyes that were clearly abnormal. It is possible that still others were more or less affected, as we judged only by conditions easily visible. In some of the descendants of this stock, indeed, ophthalmologists who have examined the eyes more thoroughly have pointed out defects which we had overlooked, and occasionally rabbits, that in their earlier months passed for normal, have later manifested defects in the lens or in other parts of the eye.

The commonest abnormality seen in both the original subjects and in their numerous descendants was partial or complete opacity of the lens (Plate I, Fig. 4), usually accompanied by reduction in size of the eye (Plate I, Fig. 2). In a few of our later strains in a different experiment, however, we have had several cases of enlargement of the eye, or *buphthalmia* (Plate I, Fig. 3). Among the rabbits I brought with me for demonstration there is one of this type which I shall be glad to have you examine. Other common defects which have appeared are cleft-iris, displacement of the lens, persistent hyaloid artery, bluish or silvery color instead of the characteristic pink of the albino eye, microphthalmia, and even almost complete disappearance of the eyeball. The cases of cleft-iris, or *coloboma,* range all the way from a narrow slit in the lower edge of the iris to a broad wedge- or U-shaped opening which amounts practically to the absence of the entire lower part of the iris. The cleft may be confined to the iris or it may extend back deeper into the eye. When one takes into account the early embryology of the eye, it is easy to see how such clefts result from failure of the choroidal fissure to close as it should do normally. The bluish or silvery color, I am told by ophthalmologists who have examined the rabbits, is due mainly to detachment of the retina. Here again, when one recalls the loose embryologic connection between the retinal layers of the eye and the outer coats, even in the

Fig. 1. Showing appearance of normal eye.

Fig. 2. Microphthalmia eye with cleft iris and opaque lens; eyeball rotated downward somewhat.

Fig. 3. Buphthalmic eye with staphylomatous sclera. The eyeball is so rotated backward that the edge of the cornea, is just visible at the upper outer angle of the lids; the lenses in both eyes are opaque.

normal eye, it is easy to see how almost any distortion of the eyeball, unevenness of growth, or accumulation of fluid might bring about such detachment.

Many of the eyes take abnormal postures (Plate I, Fig, 3). This is particularly true in some of our later strains. One or both eyes are likely to be strongly rotated downward or backward. The backward- rotation is carried to such an extreme in some cases that the cornea is visible only when the eyelids are drawn back at the outer corner (Plate I, Fig. 3), or occasionally when the animal attempts to roll its eyeball forward. In such eyes the exposed sclera in front usually bulges (*staphyloma*) and becomes transparent, simulating a cornea. When we first came across this

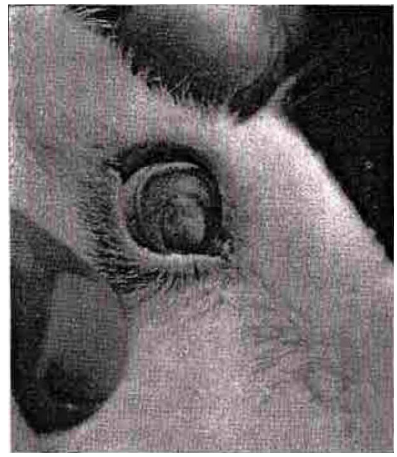

Fig. 4. Showing opaque lens and coloboma of the iris.

anomaly, in fact, we thought that we had a rabbit with a double eye on each side. I have brought one such individual with me for demonstration.

Taking into account the method of embryologic development—the relations of lens, optic cup and choroidal fissure—the defects, except those of the muscular attachment, are practically all such as might reasonably be attributed to arrests of development based upon early lens-defect. It is possible, to be sure, that we have developed antibodies against other eye-tissues as well as against the lens, since undoubtedly more or less of the aqueous humor and the vitreous body adhered to the lenses when we removed and pulped them for the original injections. Moreover, if proteins from other parts of the eye are ever in solution in the humors, they too may have been present in the antigen. Each individual protein, of course, has the capacity for engendering antibodies specific for itself. Even the lens is composed of at least four proteins: albuminoid (constituting the lens-fibers), alpha-crystallin, beta-crystallin and albumin. According to Jess and Reiss (Jess, 1920), in their study of the chemical changes which take place in cataract, alpha- and beta-crystallin, both soluble in water, make up the greater part of the lens of the young animal. These gradually decrease in quantity with age, accompanied by sclerosis—a process even more in evidence in cataractous lenses.

In some of our animals we find that an eye defective at birth, particularly if microphthalmic, may undergo further degeneration, characterized by collapse of the eyeball and resorption, so that the eyeball may eventually disappear entirely. The eyes of the mothers originally injected have always remained apparently unaffected. This is probably due to the fact that the lens-tissue of the adult is largely avascular, and that, therefore, the injected antibodies did not come into contact with it.

That the changes in the eyes of the fetuses resulted from the specific action of lens-antibodies is indicated by the fact that in the original experiment, in not one of the forty-eight controls obtained from mothers which had been treated with pure fowl-serum or with fowl-serum immunized to rabbit-tissues other than lens, was

there any evidence of eye-defects. I may add that since then, among over five hundred young obtained from mothers which are being experimented upon for other purposes with various types of sera or protein-extracts, or with typhoid bacilli, just before or during pregnancy, not a single case of eye-defect has appeared. To one familiar with the results obtained by the experimental embryologist, which show how susceptible the eye is in early embryogeny to any kind of harmful influence, the natural inclination is to regard such abnormalities as due merely to a general poisonous or inhibitive effect, rather than to specific antibodies in the blood-serum. That lens-defects may be produced by general chemical or physical means is undeniable. I know of no case yet, however, where they have become inheritable. Bagg (1922), for example, has recently found that as a result of exposure of rats to radium emanation (gamma-ray radiation) during late pregnancy, some of the young, after birth, developed eye-defects. In his paper he gives photographs of an adult in which both lenses have become opaque and the left eyelids nearly closed. As a rule, such fetally irradiated young showed other marked defects, particularly of the nervous system, and were usually sterile.

Regarding our own rabbits I can only repeat that we have never obtained the defects in question except with serum carrying specific antibodies. In any event, should the effect have originally been a general rather than a specific one, it is obvious that, germinally considered, it must sooner or later have become specific, since the anomalous eye-condition appears generation after generation without any recognizable accompanying malformations of other parts of the body.

Before passing on to the question of inheritance, I may say that by way of control, for genetical studies, in addition to what we have termed our 3A1 line, we developed another line from wholly unrelated stock, our so-called 16A1 line. Moreover, we have established still a third strain, the 84 line, which was started, not by means of fowl-serum immunized to rabbit-lens, but by the use of pulped rabbit-lens intravenously injected directly into rabbits just before or during their pregnancy. In this last case the rabbit must herself have developed antibodies against the invading lens-material. Out of eleven different females so treated, in twenty-three matings, only one individual gave us young with abnormal eyes. These defects are of the same general nature as those secured by means of fowl-serum immunized to rabbit-lens, and they behave similarly in inheritance.

As already indicated, once the defect is secured, it may be transmitted to subsequent generations through breeding (Fig. 1). So far, in the 3A1 line, we have succeeded in passing it down through nine generations. There is no reason apparent why it will not go on indefinitely, since the imperfections tend to become worse in successive generations, and also to occur in a proportionately greater number of young. The same genetical conditions hold for the other lines, although because of their more recent origin, we have manifestly not been able to carry them through so many generations.

The transmission is not infrequently of an irregular unilateral type (Fig. 1), sometimes only the right, at others only the left, eye showing the defect. In this respect it resembles genetically such anomalies as brachydactyly or Polydactyly in

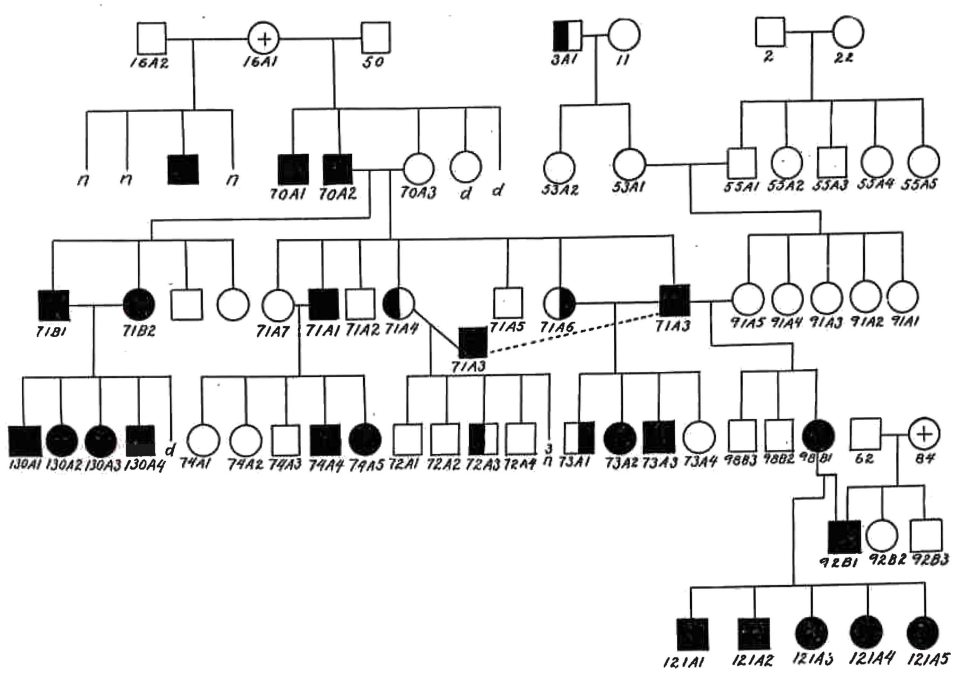

Fig. 1. Chart showing pedigree of some of the defective-eyed individuals. Only a few of the numerous matings are shown. The circle with the + sign in it indicates the female treated with lens-immunized fowl-serum (16A1) or directly with pulped rabbit-lens (84). The mother of 3A1 was treated with lens-immunized fowl-serum. Squares indicate males; circles, females; symbol all black, both eyes defective; right half black, right eye defective; left half black, left eye defective; unshaded, presumably normal-eyed; *d*, died; *n*, normal. Males 16A2, 50 and 2 (upper row) were of normal untreated stock, as were females 11 and 22. Male 62 (fourth row) was of normal untreated stock, while female 84 was injected directly with pulped lens.

man. In later generations there has been an increasing number of young with both eyes affected.

Though not analyzed completely as to its exact mode of inheritance, the abnormal condition has in general the characteristics of a Mendelian recessive. When either defective-eyed males or females are bred to normal-eyed individuals from other strains, for instance, only normal-eyed progeny result in the first generation, but the abnormal condition may be made to reappear in subsequent generations if appropriate matings are made. If we were dealing with a pair of simple Mendelian characters, the young from two individuals with the same recessive trait should all show this trait. Two of our defective-eyed rabbits, however, when bred together, are likely to produce some normal-eyed young. If, therefore, this inheritance is to be interpreted in terms of Mendelism, there is probably more than one pair of unit-factors involved.

To meet the objection that we are not getting instances of true inheritance but merely placental transmissions of antibodies or related substances from the blood-stream of the mother in each successive generation, we have established the descent

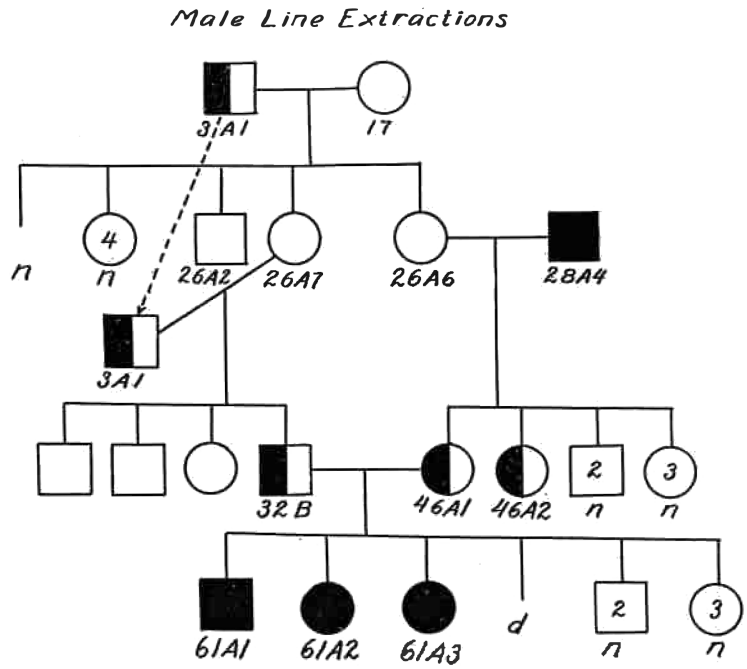

Fig. 2. Inheritance of the defects through the male line. It is plain that individuals of the 32B, 46A and 61A aeries could have derived their defects only from male ancestry originally, since female 17 was of normal and unrelated stock. Symbols same as in Fig. 1.

through the male line in a number of cases, one of which is represented in Fig. 2. To do this, females from strains of rabbits unrelated to our treated stock were mated to defective-eyed males. The first generation produced in this way was invariably normal-eyed; that is, the defective condition was recessive to normal condition. When, however, females of this generation were mated to defective-eyed males, or to normal-eyed males of similar derivation to themselves, the defects reappeared in some of the progeny, somewhat after the manner of an extracted Mendelian recessive. It is obvious that the normal condition could have been introduced into these new strains only through the germ-cells of the males, and that its transmission is, therefore, an example of true inheritance.

I feel that in establishing and developing from unrelated stock three different strains of defective-eyed rabbits—two (3A1 line and 16A1 line) by the use of fowl-serum immunized to rabbit-lens, the other (84 line) by direct injection of rabbit-lens into a pregnant rabbit—we have placed our results beyond the bounds of coincidence or chance. We can also cite further the production recently of similar lens-defect in the young of the guinea-pig, if need be, although we are not yet ready to report on this latter series of experiments.

To the biologist, perhaps the most interesting fact brought to light in these researches is the possibility of directly or indirectly inducing germinal changes by means of antibodies developed in an animal's own body against tissues taken from

individuals of the same species. Such a result together with another I have obtained in inducing the male rabbit to develop spermatotoxins against its own spermatozoa (Guyer, 1922a), lend support to the idea that an animal can build antibodies against its own tissues when these are misplaced, altered or injured, and that such antibodies may so affect the germ-cells as to induce germinal changes. Since I have discussed this point rather fully in recent papers (Guyer, 1921, 1922b, 1922c), I need not enter into it here.

LITERATURE CITED

Child, C. M. Individuality in Organisms. University of Chicago Press, 1915. The Origin and Development of the Nervous System. University of Chicago Press, 1921.

Cole, W. H. The Transplantation of Skin in Frog Tadpoles, with Special Reference to the Adjustment of Grafts over Eyes and to Local Specificity of Integument. Jour. Exp. Zool., v. 35, no. 4, May, 1922.

Fischel, A. Weitere Mitteilungen über die Regeneration der Linse. Arch. f. Entw.-Mech., v. 15, 1902. Uber rückläufige Entwicklung, Arch. f. Entw.- Mech., v. 42, 1916.

Guyer, M. F. Immune Sera and Certain Biological Problems. Am. Nat., v. 55, Mar.-Apr., 1921. Studies on Cytolyains: Experiments with Spermatotoxins. Jour. Exp. ZooL, v. 35, No. 2, Feb., 1922. Serological Reactions as a Probable Cause of Variation, Am. Nat., v. 56, Jan.-Feb., 1922. Orthogenesis and Serological Phenomena. Am. Nat., v. 56, Mar.–Apr., 1922.

Guyer, M. F., and Smith, E. A. Studies on Cytolysins: Some Prenatal Effects of Lens Antibodies. Jour. Exp. Zool., v. 26, No. 1, May, 1918. Studies on Cytolysins: Transmission of Induced Eye Defects. Jour. Exp. Zool., v. 31, No. 2, Aug., 1920.

Herbst, C. Formative Reize in der tierischen Ontogenese, 1901.

Jess, A. Die Monoaminosauren der Linsenproteine. Ztschr. f. physiol. Chem., 110, 266, 1920.

King, H. D. Experimental Studies on the Eye of the Frog Embryo. Arch. f. Entw.-Mech., v. 19, 1905.

Lewis, W. H. Experimental Studies on the Development of the Eye in Amphibia. Am. Jour. Anat., v. 3, 1904. Experimental Studies, etc. ... On the Origin and Differentiation of the Lens. Am. Jour. Anat., v. 6, 1907. Lens Formation from Strange Ectoderm in R. Sylvatica. Am. Jour. Anat., v. 7, 1907.

Speman, H. Über Correlationen in der Entwickelung dea Auges. Verh. Anat. Ges., Anat. Anz., v. 19, Erganzungsbd., 1901. Über Linsenbildung bei defekter Augenblase. Anat. Anz., v. 23, 1903. Neue Versuche zur Entwicklung des Wirbeltierauges. Verh. d. deutsch. Zool. Ges. (Stuttgart), 1908. Zur Entwicklung des Wirbeltierauges. Zool. Jahrb., Abt. f. allg. Zool. u. Physiol., v. 32, 1912.

Stockard, C. R. The Development of Artificially Produced Cyclopean Fish. Jour. Exp. Zool., v. 6, 1909. The Independent Origin and Development of the

Crystalline Lens. Am. Jour. Anat., v. 10, 1910. An Experimental Study of the Optic Anlage in Amblystoma punctatum, with a Discussion of Certain Eye Defects. Am. Jour. Anat., v. 15, 1913. The Artificial Production of Structural Arrests and Racial Degeneration. Proc. N. Y. Path. Soc., N.S., v. 13, 1914. Developmental Rate and Structural Expression: an Experimental Study of Twins, Double Monsters and Single Deformities, etc. Am. Jour. Anat., v. 28, 1921.

Wachs, H. Neue Versuche zur Wolff'schen Linsenregeneration. Arch. f. Entw. -Mech., v. 39, 1914.

Werber, E. I. Critical Notes on the Present Status of the Lens Problem. Biol. Bull., v. 34, No. 4, April, 1918.