CYBERNETIC ORIGINS OF REPLICATION

DAVID P. BLOCH*
Botany Department, University of Texas, Austin, TX 78713, U.S.A.

(Received 2 March, 1987)

Abstract. An evolutionary progression leading toward replication is resolved into several phases; (a) the replication of RNA segments by self-priming and -templating, (b) the replication of single stranded molecules by elongation and controlled scission, (c) replication of complementary duplexes and (d) replication of DNA. The initial phase is suggested by evidence for the existence of tandem repeats in an early population of molecules presumed to be ancestral to today's structurl RNAs. Relics of these repeats are seen in the positioning of sequence matches between transfer and ribosomal RNAs. Conservation of the positions of the matches is indicated by persistence of a periodicity in their spacings along the molecules.

Selection is viewed as a vector, with a source and a focus. The evolutionary progression entails shifts in the source of selection, from external catalysts to the replicating molecule itself, and in its focus, from substrate to replicator, to the products of the replicator's activity. When the source and focus of selection are the same selection becomes internalized, and replication and Darwinian evolution follow.

Catalytic specificity is regarded as an antecedent to natural selection. Shifting of the source and focus of selection and switches in evolution's 'vehicle', the most fundamental thing that evolves, result in profound changes in the modes of evolution. Control provides a conceptual framework within which entry into a Darwinian mode of evolution, and ultimately liberation from Darwinian evolution might be explained.

1. Introduction

DNA replication depends on support systems whose synthese require transcription and translation of information from the DNA itself. This complex process must have evolved by way of small steps whose evolutionary intermediates functioned differently from the system that works today. The earliest replicating molecules were probably autonomous and the early catalysts probably exogenous, possibly mineral in origin. Later elaborations must have involved partitioning of functions among specialized molecules.

Clues to the nature of the early molecules have been deduced from the kinship found between structural RNAs and transfer RNAs of contemporary organisms. The large number of matching bases found between the two classes of RNAs are considered to be sequence homologies, reflecting the common ancestry of the molecules (Bloch *et al.*, 1983). A spectral analysis of the homologies plotted on a standard frame of reference indicates a principal period of 9 (Nazarea *et al.*, 1985).

* This paper was prepared for posthumous publication by H. S. Forrest and M. P. Staves. Reprint requests should be addressed to M. P. Staves, Dept. of Biochemistry, University of Alabama at Birmingham, AL 35294, U.S.A.

Origins of Life and Evolution of the Biosphere **18** (1988) 87–96.

It is thought that these periods reflect the tandem 9 base repeats in the ancestral molecules. A model for the generation of ancestral molecules was proposed by Bloch *et al.* (1985). An early string of 9 bases underwent a succession of elongations through self-priming and templating to give an intermediate of 72 bases capable of folding into a cloverlead. Further cycles of replication led to longer molecules. A population of such molecules later underwent recombination yielding composite molecules.

The earliest events leading to replication probably involved simple autonomous systems rather than mutually interdependent networks. The informational molecules at the earliest stages are thought to have been RNA rather than DNA because of RNA's versatility as a replicator (Spiegelman *et al.*, 1968) and a catalyst (Kruger *et al.*, 1982; Guerier-Takada and Altman, 1984), and its direct involvement in the manipulation of genetic information.

The present work proposes a series of processes beginning with chemical systems and working toward replicating systems that have the requisites for Darwinian evolution. The model describes events that introduce new elements of control, such as are also experienced during Darwinian evolution. The progression provides a conceptual framework, bridging the gap between pre-Darwinian chemical processes and Darwinian evolution.

2. Shifts in Selection's Vectors

The approach is cybernetic. The proposed scheme for entry into a Darwinian mode invokes the chance introduction of new sources of control of and new modes of response by the systems that are controlled. Similar events also occur in well developed Darwinian systems. The evolutionary paradigm recognizes several variables that involve selection and evolution's vehicle. The term 'selection', otherwise used in a traditional sense is perceived as a vector with a source and a focus. In present day systems an important source of selection is the environment. Selection's focus is on the individual organism by way of its phenotype. Changes that become established increase fitness as measured by reproductive success, or at least they are neutral (Kimura, 1968). The focus of selection may change. Various treatments have placed the focus of selection on DNA (Doolittle and Sapienza, 1980; Orgel and Crick, 1980), genes (Dawkins, 1976), the individual organism (Darwin, 1859), kin (Maynard-Smith, 1964, 1976), group or species (Wynne-Edwards, 1962). The early history of evolution may have been in part the shifting of the focus of selection from the replicator itself to more remote effects of the replicator's activity (Dawkins, 1982).

Selection's source may shift from one part of the environment to another as circumstances change, or may be internalized as in some aspects of sexual selection (Darwin, 1859) and in various kinds of selection, conscious or otherwise, practiced by humans on their own kind. This internalization equates source and focus, and has accompanied some profound innovations in the ways in which evolution works.

It has been argued (Alexander, 1979) that selection that appears to act at levels higher or lower than the individual organism might best be treated as acting directly on the individual organism, i.e., inclusive fitness (Hamilton, 1964a, b). We consider here the individual organism as one of several links through which selection acts on what replicates. In any event it must be recognized that elements at many levels enter into the mechanics of the selection process as it acts on individuals. A broad interpretation of selection permits a particularization that can be used to advantage. We are here interested in tracking the roles that selection and its evolutionary antecedents play in the transitions from growth of molecules to replication of molecules, and ultimately to more remote adaptations that facilitate replication, as selection shifts form one level to another.

3. Evolution's Vehicle

Another variable is evolution's vehicle, the most fundamental thing that evolves. If RNA-based organisms were the direct or indirect progenitors of DNA-based organisms, RNA to DNA would be an example of a vehicle switch. If Cairns-Smith's (1982) take-over hypothesis is correct, and the 'organisms of the first kind' (Cairns-Smith, 1979) were clays (see also Weiss, 1981), clay-based to nucleic acid-based evolution would be a vehicle switch. If it is so that changes in human culture and technology can occur independently of continuing changes in humans DNA, DNA to culture/technology would be a switch in vehicles.

4. The Sequence

Table I describes a sequence in which earlier steps set the stages for later ones, following an approach similar to that of Schuster (1981). The progression tracks a path from growth to replication and includes elaboration of phenotypes. The table points out changes in selection's vectors and switches in evolution's vehicle. The sequence attempts to assimilate within a Darwinian framework the origin of the tandem repeats suggested by the analyses cited above.

Step I describes a simple second order reaction, the condensation of two molecules under thermodynamically favorable conditions. An example might be the condensation of an organic acid and an alcohol to form an ester under dehydrating conditions.

Step II: If the reaction is facilitated by a catalyst, rate control shifts from concentration of the reactants to the catalyst, its binding constants and activation energy. If there are competing reactions and time constraints the product will depend on specificity or selectivity of the catalyst. Specificity would be an antecedent of natural selection but would be dead-ended evolutionarily because of lack of mechanisms for feedback leading to self-perpetuating change.

Step III is of greater biological significance. Condensation of bifunctional molecules leads to the formation of polymers, the elongation of molecules by adding

TABLE I

Summary of the sequence

	Precursor	Chemical process	Result	Consequences of biological importance	Controlling factors	Cybergenic* events
I	mono-functional molecules	condensa – tion	condensed product		activation energy, reactant concentration	introduction of catalyst
II	,,	,,	,,	selectivity, specificity	catalysis	
III	bifunction-al mole – cules	polymer-ization	homo-polymers	growth	,,	
IV	polyfunc-tional mole – cules	,,	random hetero-polymers	growth of in-formational molecules		
V	ribo – nucleo-tides	self-pri-ming and templat-ing. copying	tandem re-repeated hetero-polmers, indefinite length	replica-tion of information segments	exogenous & endogenous catalysts, cycles	participation of product in control of replication
VI	,,	random breakage	above poly-mers of limited but unde-fined length	prolifer-action of inform-ation	suscept-ibility to breakage	internalization of selection's source
VII	,,	selective cleavage	,, defined length	replication of inform-ational molecules	specific cleavage sites	
VIII	,,	separa-tion of priming and tem-plating functions	complement – ary duplex	fidelity of replica-ation. recombination	,,	
IX	ribo-nucleo-tides, amino acids	separation of repli-cator from its product	proteins	facili-tation of repli-cation, translation	exogenous catlysts of endogen-ous origin	extension of phenotype from replicator to product
X	,, and deoxy-nucleo-tides	separation of replic-ation from coding	DNA	stability transcription	switching evolution's vehicle	switching evolution's vehicle, RNA → DNA

* Cybergenic event is defined as an event that introduces new elements that become established.

onto a priming end. A consequence is molecular 'growth'. The driving forces would be mass action, the availability and concentration of reactants and the efficacy of catalysts. A biological example is the polymerization of adenylate to form polyadenylic acid. If the existence of a preformed polymer can facilitate orientation of the reactants through a crystallization-like process a precondition for self-replication is met.

Step IV is the formation of heteropolymers from a heterogeneous mixture of polyfunctional molecules. The process is similar to homopolymerization but would have the potential for creating information and would establish a precondition for a subsequent step, the copying of information. If the linkage of the different reactants reflect only availability it is likely that a random polymer would be formed. If certain ones of the reactants are polymerized with greater rates than others, these would be favored.

Step V: Selection for preferred sequences might reflect external factors such as catalytic surfaces resulting in external 'instruction' (determination of sequence in an information-bearing molecule, Biebricher *et al.*, 1981a, b). An influence of preformed polymers on new polymers would result in self-instruction. A molecule's nearest potential source of instruction would be self or a recent copy of self. Looping back and self-copying or adding on to an end of priming molecules partially paired with the template (Goulian *et al.*, 1968; Schuster, 1981) could duplicate information during growth in the length of the molecules but would not increase numbers of molecules.

In terms of selection's vector, the source of instruction shifts from external catalytic agents to the molecule itself, representing a shift in the origin of specificity. The focus remains the molecule. Here the shift represents an internalization of control. The closest present day biological model illustrating this switch would be the change from enzyme instruction to template instruction following the *de novo* synthesis of RNA using Qβ viral replicase (Biebricher *et al.*, 1981a, b, 1986). Steps III through VI are telescoped in the Qβ experiments. Here incipient selection based initially on enzyme specificity or substrate selection comes to fruition in a Darwinian sense. Replication of information occurs, but not replicating of molecules.

The process may have several requirements. One would be an alternating environmental cycle that at one time promotes copying, at another, restoration of the conditions enabling another round of elongation. If initiation is dependent on a configuration such as a hairpin then the process should be sequence dependent. Sequence dependence would in turn confer an advantage to molecules with the ability to copy the required or preferred sequence. Mutually supportive elements such as a complementary 5′ end (see discussion) would tend to establish and maintain a successful initiating sequence. A set of basic conditions for selection of information would exist from which the system could not stray very far. Most deviants would work poorly and be selected against. The discussion proposes some mechanisms by which selection for self-priming and templating would be favored.

Step VI: The above process would reproduce information by replicating sequences

in molecules of indefinitiely increasing length. An increase in the number of information bearing molecules might occur through breakage as stability decreases with increasing length. Random breakage would slow the process by forming ends that make less efficient primers.

Step VII: Selection could confer an advantage to molecules with specific breaking points (Kruger *et al.*, 1982; Guerrier-Takada and Altman, 1984), if these occurred near the priming ends. The resulting elongation and breakage cycles would reproduce discrete molecules rather than numbers of segments with a molecule.

Step VIII: Separation of the priming and template functions among different molecules would approximate the modern system of doule stranded complementary sequences. A consequence might be increase in fidelity in length and sequence.

Step IX is a large jump. The separation of functions of replicator and catalyst leads to the introduction of phenotype in its current usage. Separation would necessitate the introduction of translation machinery and may have involved a confluence of the RNA system with another based on proteins.

Step X. Separation of the functions of replication from RNA to DNA would result in the switch of evolution's vehicle from RNA to DNA and would require the introduction of a system for transcription.

5. The Evolution of Replication Resolved into Phases

The progression leading to replication is resolved into several phases. An early one leads to replication of segments within a molecule by self-priming and templating by a single strand. A later phase is the replication of molecules by breakage of the elongating strands. Still later, following separation of templating and priming functions to different molecules, replication of a complementary duplex would occur.

There are advantages to replication by elongation of single molecules. If a string of bases arises that is good at copying, perhaps because it contains an efficient initiating sequence near its 3′ end, elongation by copying other templates would result in burying the priming sequence in the middle of the molecule. If the sequence itself acts as template and is copied onto other priming ends, the favored sequence (or its complement) would be incorporated into a molecule whose own 5′ end would not have the complement of the initiating sequence. The integrity of the priming sequence could be maintained in a process in which the copier and the copied are one and the same molecule. Every round of self-elongation would restore the original priming end (Figure 1). Self-copying would also make economic sense. It would obviate the need for a partner and provide for elongation of unique molecules in low concentrations. Multiple cycles of copying would lead to the tandem repeats whose early existence is suggested by the periodicities in spacing of the homologies among the different classes of RNAs (Bloch *et al.*, 1985; Nazarea *et al.*, 1985).

The introduction of controlled breakage through exogenous catalysts or through autocatalytic scission (Kruger *et al.*, 1982; Guerrier-Takada and Altman, 1984)

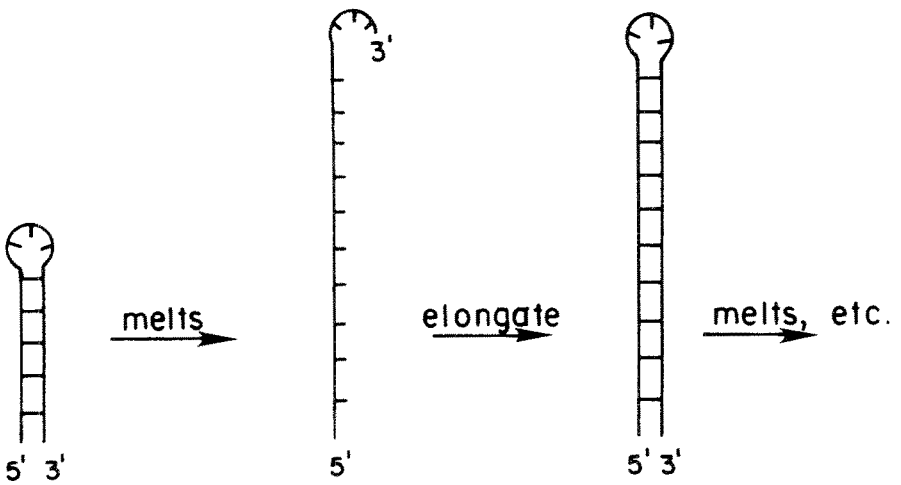


Fig. 1. Schematic showing the complementary relationship between the 3′ and 5′ ends essential for maintaining a preferred 3′ sequence during successive round of replication.

would provide an escape from indefinite elongation and consequent strain-induced breakage. Strain breakage by itself would likely be random and would not regenerate priming ends. Catalytic breakage coupled with strategically placed breakpoints could maintain these ends.

An eventual separation of priming and templating functions among different molecules leading to replication of a double helix should increase fidelity in copying the lengths and sequences of the replicating units. This is a large leap and no attempt is made here to propose mechanisms that might bridge the gap.

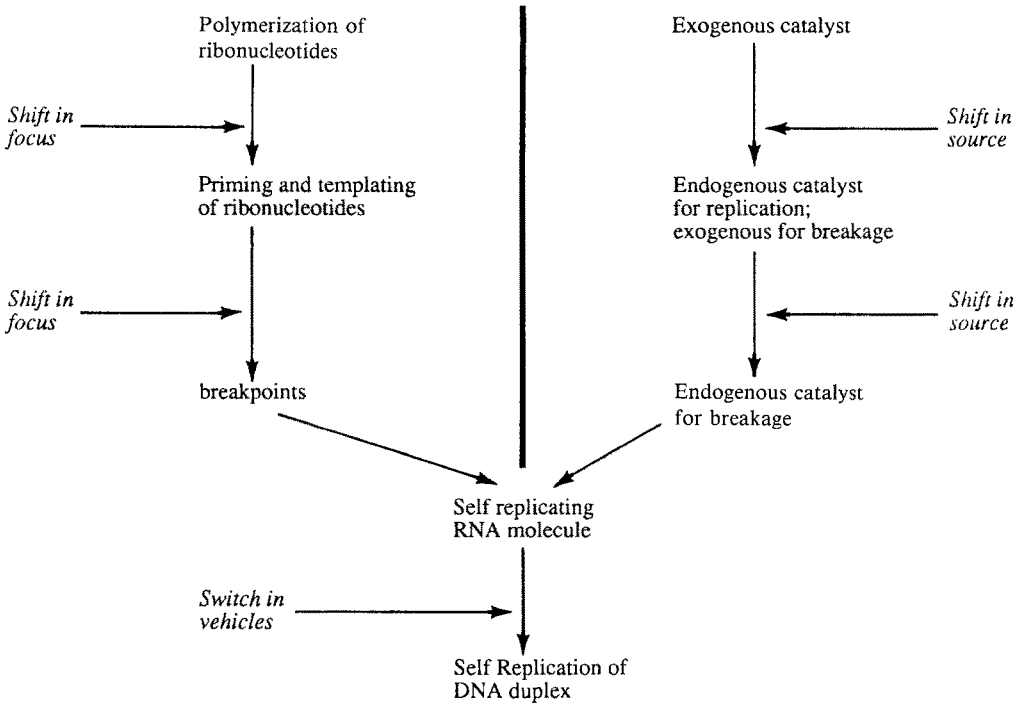
6. Shifts in Control Leading to Replication

The progression, condensation to polymerization to replication is described in terms of introduction of new elements of control. We envision shifts in the determiners of the course of these reactions from concentrations of reactants to catalysts, perhaps mineral surfaces, to the products themselves sharing with the exogenous catalysts a role in their own replication.

The focus of control during the earliest stages of this series is on the reactants themselves. The reactants are portrayed as increasing in complexity as a conceptual device. No mechanisms are implied for establishing causal links or temporal relationships among the early steps. While bifunctional and polyfunctional molecules may have preceeded one another and set the stage for the higher level of complexity, a link leading from one step to another could not have resulted from Darwinian selection. It is only when heteropolymers were achieved that catalytic

TABLE II

Diagrammatic representation of shifts of selection's vectors (source and focus) and evolution's vehicles, during evolution



selectivity or specificity could yield to Darwinian selection with its predilection for direction in an evolutionary sense.

Table II depicts the shifts in the source and focus of control during two phases of the progression.

At this stage when there is the first merging of the source and focus of selection, an internalization of control, simple Darwinian selection appears to be possible.

7. Shifts in Control Leading to Phenotype

The introduction of controlling elements, both new sources of control and new foci for its effects, can occur at a variety of levels – gene, protein, cell surface, behavior. It is no large leap from consideration by the effects of such events *within the framework of selection* to its effects *on* selection. In the interplay between evolution and control the roles of each as cause and effect can be reversed. The proposed series in Table I and II leading to replication, is one in which changes in control are antecedent to evolution but are not necessarily determining.

The effects on the selection process brought about by the introduction of an element of control may be profound. When the focus shifts from a replicator to some consequence of the replicator's activity that feeds back on replication, the status of 'phenotype' is lifted from a direct reflection of replicating ability to the effects of some agent that indirectly feeds back on the process. Changes in control here bring about an evolutionary innovation, phenotype.

Phenotype is usually thought of as the effect of gene expression mediated by a gene product – an avenue for the environment to screen the genome for fitness. In current usage at the molecular level, phenotype is often due to an effect of an enzyme activity. Because of the circuitous route selection may take it is useful to include indirect extensions of gene activity as phenotype. A gene mutation in one organism may invoke a response in a second organism that interacts with the first (Dawkins' 'extended phenotype', 1982). Conversely, the genotype – phenotype link can be contracted to include only the properties of the gene (Spiegelman *et al.*, 1968). The recent descriptions of catalytic activity in RNAs (Kruger *et al.*, 1982; Guerrier-Takada and Altman, 1984) suggest the possibility of replication and phenotype as phenomena residing in the same molecule. A polymer that directs its own synthesis or the synthesis of its complement, is both source and focus of selection.

8. Vehicle Switch in Evolution

If RNA was an early replicator, the fact that DNA plays that role today points up the fact that evolution switched vehicles somewhere along the line. Little can be said of the mechanism of transfer. Its consequence may only be guessed at in the absence of information about RNA as a replicator under comparable circumstances. Other examples of vehicle switches were alluded to earlier and will be mentioned again below.

9. Evolution as a Discontinuous Process

Although evolutionary trends may be understandable in retrospect they are hardly predictable in their details. Certainly the pathways are not. Discontinuity is inherent in the process. The source from which control comes is often unrelated to the focus of its effects which makes establishment of conceptual links difficult. This discontinuity offers a clue to the nature of the gap between chemical and biological evolution. With regard to control, its manipulation and use, the events that mark the prebiological stages are not very different from those that operate in the biological. An event that achieves the requisite for the Darwinian process is the internalization of selection – yielding an entity that is both origin and target of selection. This internalization might well mark the onset of evolution by natural selection and was to occur repeatedly at successive levels as evolutionary systems increase in complexity. At the other end of the process another switch in vehicles from DNA to culture/technology based evolution marks a switch in vehicles and liberates evolution

from its Darwinian constraints. This is a similar invention, marked by discontinuity and by internalization of selection. It is reminiscent of the invention of protein catalysts that facilitate replication, and of the proposed shift in selection's focus leading from replication of segments to replication of molecules.

Acknowledgements

This work was supported by grants from the RGK Foundation and the Richard Lounsbery Foundation.

References

Alexander, R. D.: 1979, *Darwinism and Human Affairs*, University of Washington Press, Seattle.
Biebricher, C. K., Eigen, M., and Luce, R.: 1981a, *J. Mol. Biol* **148**, 369–390.
Biebricher, C. K., Eigen, M., and Luce, R.: 1981b, *J. Mol. Biol.* **148**, 391–410.
Biebricher, C. K., Eigen, M., and Luce, R.: 1986, *Nature* **321**, 89–91.
Bloch, D. P., McArthur, B., and Mirrop, S.: 1985, *Biosystem* **17**, 209–225.
Bloch, D. P., McArthur, B., Widdowson, R. B., Spector, D. Guimaraes, R. C., and Smith, J.: 1983, *J. Mol. Evol.* **19**, 420–428.
Bloch, D. P., McArthur, B., Widdowson, R., Spector, D., Guimaraes, R. C., and Smith, J.: 1984, *Origins of Life* **14**, 571–578.
Cairns-Smith, A. G.: 1979, *Chemistry in Britain* **15**, 576–579.
Cairns-Smith, A. G.: 1982, *Genetic Takeover and the Mineral Origins of Life*, Cambridge University Press, London.
Darwin, C.: 1859, *The Origin of Species by Means of Natural Selection*, First ed. Reprinted by Random House Inc., New York.
Dawkins, R.: 1976, *The Selfish Gene*, Oxford University Press, New York.
Dawkins, R.: 1982, *The Extended Phenotype. The Gene as the Unit of Selection*, W. H. Freeman and Co., New York.
Doolittle, W. F. and Sapienza, C.: 1980, *Nature* **284**, 601–603.
Goulian, M. J., Lucas, L., and Kornberg, A.: 1968, *J. Biol. Chem.* **243**, 627–638.
Guerrier-Takada, C. and Altman, S.: 1984, *Science* **223**, 285–286.
Hamilton, W. D.: 1964a, *J. Theoret. Biol.* **7**, 1–16.
Hamilton, W. D.: 1974b, *J. Theoret. Bio.* **7**, 17–52.
Kimura, M.: 1968, *Nature* **217**, 624–626.
Kruger, K., Grabowski, P. J., Zaug, P. J., Sands, J., Gottshling, D. W., and Cech, T. R.: 1982, *Cell* **31**, 147–158.
Maynard-Smith, J.: 1964, *Nature* **201**, 1145–1147.
Maynard-Smith, J.: 1976, *Quart. Rev. Biol.* **51**, 277–283.
Nazarea, A., Bloch, D. P., and Semrau, A.: 1985, *Proc. Nat. Acad. Sci. USA* **82**, 5337–5341.
Orgel, L. E. and Crick, F. H. C.: 1980, *Nature* **284**, 604–607.
Schuster, P.: 1981, 'Prebiotic Evolution', in H. Gutfreund (ed.), *Biochemical Evolution*, Cambridge University Press, Cambridge.
Spiegelman, S., Pace, N. R., Mills, D. R., Levisohn, R., Eikhorn, T. S., Taylor, M. M., Peterson, R. L., and Bishop, D. H. L.: 1968, *Cold Spring Harbor Symp. Quant. Biol.* **33**, 101–124.
Weiss, A.: 1981, *Angew. Chem. Int. Ed. Engl.* **20**, 850–860.
Wynne-Edwards, V. V.: 1962, *Animal Dispersion in Relation to Social Behavior*, Oliver and Boyd, Edinburgh.