therapeutic drugs, together with many other measures, in order to keep the immense number of pathogenic organisms under control.

1.3.4.3. *Non-communicable diseases*

Of this large and diverse category of human afflictions we have already touched upon genetic disorders in Section 1.3.3 above. Other major types of non-communicable diseases include cancer, aging disorders, diabetes, arthritis, and cardiovascular and neurological illnesses. The field of non-communicable diseases is immense. Describing in any detail the current projects in, and potential impact of, protein and nucleic acid crystallography on these diseases would need more space than this entire volume on macromolecular crystallography. Hence, only a few selected examples out of the hundreds which could be described can be discussed here. Table 1.3.4.5 lists many examples of human protein structures elucidated without any claim as to completeness – it is simply impossible to keep up with the fountain of structures being determined at present. Yet, such tables do provide, it is hoped, an overview of what has been achieved and what needs to be done.

1.3.4.3.1. *Cancers*

Over a hundred different cancers have been described and clearly the underlying defect, loss of control of cell division, can be the result of many different shortcomings in different cells. The research in this area proceeds at a feverish pace, yet the development, discovery and design of effective but safe anti-cancer agents are unbelievably difficult challenges. The modifications needed to turn a normal cell into a malignant one are very small, hence the chance of arriving at 'true' anti-cancer drugs that exploit such small differences between normal and abnormal cells is exceedingly small. Nevertheless, such selective anti-cancer agents would leave normal cells essentially unaffected and are therefore the holy grail of cancer therapy. Few if any such compounds have been found so far, but cancer therapy is benefiting from a gradual increase in the number of useful compounds. Many have serious side effects, weaken the immune system and are barely tolerated by patients. However, they rescue large numbers of patients and hence it is of interest that many targets of these compounds, proteins and DNA molecules, have been structurally elucidated by crystallographic methods – often in complex with the cancer drug. The mode of action of many anti-cancer compounds is well understood, *e.g.* methotrexate targeting dihydrofolate reductase, and fluorouracil targeting thymidilate synthase. These are specific enzyme inhibitors acting along principles well known in other areas of medicine. Several anti-cancer drugs display unusual modes of action, such as:

(*a*) the DNA intercalators daunomycin (Wang *et al.*, 1987) and adriamycin (Zhang *et al.*, 1993);

(*b*) cisplatin, which forms DNA adducts (Giulian *et al.*, 1996);

(*c*) taxol, which not only binds to tubulin but also to bcl-2, thereby blocking the machinery of cancer cells in two entirely different ways (Amos & Lowe, 1999);

(*d*) camptothecin analogues, such as irinotecan and topotecan, which have the unusual property of prolonging the lifetime of a covalent topoisomerase–DNA complex, generating major road blocks on the DNA highway and causing DNA breakage and cell death;

(*e*) certain compounds function as minor-groove binders, *e.g.* netropsin and distamycin (Kopka *et al.*, 1985);

(*f*) completely new drugs which were developed based on the structures of matrix metalloproteinases, purine nucleotide phosphorylase and glycinamide ribonucleotide formyltransferase and which are in clinical trials (Jackson, 1997).

Meanwhile, it is sad that crystallography has not yet made any contribution to the molecular understanding of multi-drug resistance in cancer. The resistance is caused by cellular pumps that efficiently pump out the drugs, often leading to failed chemotherapy (Borst, 1999). On the other hand, the structures of major oncogenic proteins such as p21 (DeVos *et al.*, 1988; Pai *et al.*, 1989; Krengel *et al.*, 1990; Scheffzek *et al.*, 1997) and p53 (Cho *et al.*, 1994; Gorina & Pavletich, 1996) are of tremendous importance for future structure-based design of anti-neoplastic agents.

1.3.4.3.2. *Diabetes*

The hallmark characteristic of type I diabetes is a lack of insulin. A major therapeutic approach to this problem is insulin replacement therapy. Unfortunately, the insulin requirements of the body vary dramatically during the course of a day, with high concentrations needed at meal times and a basal level during the rest of the day. Only monomeric insulin is active at the insulin receptor level, but insulin has a natural tendency to form dimers and hexamers that dissociate upon dilution. Thanks to the three-dimensional insight obtained from dozens of insulin crystal structures, as wild-type (Hodgkin, 1971), mutants (Whittingham *et al.*, 1998) and in complex with zinc ions and small molecules such as phenol (Derewenda *et al.*, 1989), it has been possible to fine-tune the kinetics of insulin dissociation. The resulting availability of a variety of insulin preparations with rapid or prolonged action profiles has improved the quality of life of millions of people (Brange, 1997).

1.3.4.3.3. *Blindness*

The main causes of blindness worldwide are cataract, trachoma, glaucoma and onchocerciasis (Thylefors *et al.*, 1995). Trachoma and onchocerciasis are parasitic diseases that destroy the architecture of the eye; they were discussed in Section 1.3.4.1. The other two are discussed here. During cataract development, the lens of the eye becomes non-transparent as a result of aggregation of crystallins, preventing image formation. Crystal structures of several mammalian beta- and gamma-crystallins are known, but no human ones yet. In glaucoma, the optic nerve is destroyed by high intra-ocular pressure. One way to lower the pressure is to inhibit carbonic anhydrase II, a pivotal enzyme in maintaining the intra-ocular pressure. On the basis of the carbonic anhydrase crystal structure, researchers at Merck Research Laboratories were able to guide the optimization of an *S*-thienothiopyran-2-sulfonamide lead into a marketed drug for glaucoma: dorzolamide (Baldwin *et al.*, 1989).

1.3.4.3.4. *Cardiovascular disorders*

Thrombosis is a major cause of morbidity and mortality, especially in the industrial world. Hence, major effort is expended by pharmaceutical industries in the development of new classes of anti-coagulants with fewer side effects than available drugs, such as heparins and coumarins. Because blood coagulation is the result of an amplification cascade of enzymatic reactions, many potential targets are available. At present most of the effort is directed towards thrombin (Weber & Czarniecki, 1997) and factor Xa (Ripka, 1997), responsible for the penultimate step and the step immediately preceding it in the cascade, respectively. Thrombin is especially fascinating owing to the presence of at least three subsites: a primary specificity pocket with the catalytic serine-protease machinery, an exosite for recognizing extended fibrinogen and an additional pocket for binding heparin. This knowledge has led to the design of bivalent inhibitors which occupy two sites with ultra-high affinity and exquisite specificity. Several of these agents are in clinical trials (Pineo & Hull, 1999).

references