Regular Review

The polymerase chain reaction: a tool for molecular medicine

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The polymerase chain reaction has been unquestionably unique, as new techniques go, in the speed with which it has been embraced by non-experts in most specialties of the biological sciences, including medicine. The reason for this is the unusual simplicity of the procedure. In terms of its power to drive biological research, the advent of the polymerase chain reaction can certainly be compared with the discovery of the techniques of molecular cloning some 20 years ago. However, whereas years of training and practice were usually needed to master the many and complex skills of recombinant DNA technology, the complete beginner can start to perform polymerase chain reaction experiments and generate meaningful results within a few days at most—hence the explosion of activity.^{1-1c}

The technique was first described in its initial format in 1985,² and over the next three years appreciation of its potential gradually became widespread. This potential was fully realised in about 1988. It coincided with the commercial development of two key components for the polymerase chain reaction: a DNA polymerase that could be heated at quite high temperatures (boiling water) without losing its activity, and robust machines that would quickly heat and cool samples repeatedly in a cyclic fashion.³ Synthesis of the oligonucleotides required as primers in the reaction had already become a commonplace procedure. In the past four years the reaction has become probably the most widely used single technique in all branches of the biological sciences.

What is the polymerase chain reaction?

The polymerase chain reaction is a delightfully simple concept, first alluded to 30 years ago,⁴ and very reminiscent of the way that cells duplicate their DNA to expand their numbers *in vivo*. The other simple analogy is to the chain reaction of nuclear physics. The technique permits the analysis of nucleic acids (DNA or RNA) from any source.

PROCEDURE

A small sample of DNA in solution is placed in a single tube. (As will become clear below, any RNA samples for analysis are simply converted to DNA in a single preliminary step.) Two oligonucleotides (which are easy to make artificially on automatic machines or can be purchased from several suppliers) are added to the tube. Their sequences are chosen so that they match two short DNA sequences that flank the region of interest. The exponential increase in the amount of DNA sequence that is now known, and the universal accessibility of this data through computer databases, mean that any scientist can quickly start to study genes

Outline of the procedure

- A small sample of DNA is placed in a tube
- Two oligonucleotides are added. These have sequences matching two sequences of the DNA that flank the region of interest
- A thermostable DNA polymerase is added
- The mixture is heated to just below 100°C and the DNA dissociates into two single strands
- The solution is allowed to cool and the single strands bind to the oligonucleotides, which are in excess
- The oligonucleotide now acts as a primer for DNA polymerase and is extended to form a new double stranded molecule
- The cycle is repeated, with the amount of DNA doubling each time

of interest in his or her own laboratory. There is no need to obtain DNA clones from other workers, so all the problems previously associated with that are avoided.

Considerably more oligonucleotides than the DNA to be analysed are provided deliberately. A thermostable DNA polymerase is added to the same tube.⁵ Deoxynucleoside triphosphates (dNTPs) and salts and buffer to allow the enzyme to work properly are also included. All these reagents are available commercially. Taq DNA polymerase was isolated from the thermophilic organism, *Thermus aquaticus*. Not only can it tolerate heating at 100°C, but it makes DNA at high temperatures compared with the 37°C physiological temperature optimum of most enzymes. A cloned version of this enzyme without any nuclease activity is also available.

This simple mixture is placed in a heater and the temperature quickly raised to just below the boiling point of water. This causes the double stranded DNA in the sample to dissociate into two single strands as the hydrogen bonds, which hold the two strands together under physiological conditions, break down reversibly on heating. After a minute or so, the solution is allowed to cool towards physiological temperature. This allows hydrogen bonds to re-form. The two DNA strands in the sample would, of course, usually relocate their partners and re-form the paired double helix. However, in the polymerase chain reaction tube the oligonucleotides, which are present in great excess, quickly and highly specifically bind to their complementary single strands from the denatured sample. As soon as this happens the oligonucleotide can act as a primer for DNA polymerase and is extended to form a new double stranded molecule. Thus each double stranded DNA molecule in the original sample has been melted to

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form two single stranded molecules, which have then been turned into two double stranded molecules.

There is now twice as much double stranded sample DNA present in the tube as there was to start with. The cycle is then repeated and the amount of sample DNA doubled further with every cycle. This geometrical amplification is perfectly analogous to a nuclear chain reaction with 2-fold, 4-fold, 8-fold, 16-fold, 32-fold, etc amplification at subsequent steps. After n cycles the degree of amplification is of course 2^n . Thus after 10 cycles 1024-fold amplification is achieved and after 20 cycles 10⁶-fold amplification results.

No great manual dexterity is required to perform the technique, in that all these reactions go on by simply automatically heating and cooling without ever opening the reaction tube. The whole process is outlined in figure 1.

The orientation of the two strands in double stranded DNA is important to note. They are described as being antiparallel, which means that when bound together in a helix one reads 5' to 3', the other 3' to 5'. This simple consideration dictates the design of the synthetic oligonucleotides. When making double stranded DNA, Taq polymerase attaches new residues to the 3' end of primers. Thus in the polymerase chain reaction DNA is essentially copied only between the two primers. After multiple cycles of amplification, the predominant double stranded DNA species in the sample is a fragment whose two ends are defined by the

two primer oligonucleotides—that is, not all the DNA in the original sample is amplified. For example, if the sample DNA were total human DNA from a blood sample, and therefore contained 3×10^9 base pairs, amplification of any 300 base pair segment could be specifically achieved. It will be appreciated that the signal to noise problems inherent in detecting and analysing 300 base pairs out of 3 billion (1 in 10⁷) are very much alleviated after 30 cycles of polymerase chain reaction, when 10⁹ copies of the 300 base pair fragment are present for every single copy of the total genomic DNA background.

PROBLEMS

The polymerase chain reaction is not quite infinitely powerful or infallible. Eventually, after many cycles, the concentration of oligonucleotide primers falls because they have all been incorporated into products of the reaction. Similarly, the concentration of deoxynucleoside triphosphate substrates, and indeed the activity of the DNA polymerase itself, decline. Products of pyrophosphate breakdown may inhibit further reaction. Amplification will usually be sufficient for most analytical purposes by this stage, but should even greater sensitivity be necessary—for example, in the analysis of DNA from a single cell—then a tiny aliquot from the first polymerase chain reaction tube (as a source of sample DNA) is simply transferred to a second identical tube, and amplification is continued. Should specificity be a problem (for instance, if the target DNA comes in a sample that also contains many other closely related DNA sequences) then this can be overcome using “nested” primers in the second tube. These match sequences just inside the two original primer sites. Thus nested amplification of a product of the polymerase chain reaction depends overall on accurate recognition by four independent oligonucleotides.

The other aspect that may cause problems is the specificity of the priming reaction itself. This depends on several considerations. The size of the oligonucleotide will determine whether it occurs more than once in a sample DNA and therefore might prime DNA polymerase activity at multiple sites. This will generate only spurious products of the polymerase chain reaction when unwanted priming also occurs close by on the other DNA strand. However, the reaction may be inefficient if one of the primers is depleted because of excessive spurious priming. Several simple measures can be taken to eliminate this type of problem should it arise. These include using raised temperatures during cycling, adjusting the magnesium concentration (which partly determines the ease of hybridisation), and various other easy tricks to destabilise partially mismatched primers. On balance, these considerations do not usually constitute a serious problem, and any teething troubles can usually be overcome quickly by minor and obvious adjustment to reaction conditions.

Why has the reaction had such a major impact?

SOUTHERN BLOTTING

The impact of the polymerase chain reaction can be attributed to its practical simplicity and to its speed, sensitivity, and specificity. Consideration of some of the routine day to day techniques of molecular biology allows this to be illustrated. In a genomic Southern blot it is impractical to load more than 10 μ g (10^{-6} g) of restriction enzyme digested DNA per lane of an agarose gel. Higher loading causes overloading and streaking. If one is interested in a 3 kilobase fragment then this constitutes about 10 pg (10^{-12} g) of the sample. Detecting 10 pg of anything is taxing, and doing so when it is effectively “contaminated” with a million-

FIG 1—Schematic illustration of the polymerase chain reaction. Initially runoff priming occurs. In subsequent cycles the product with ends defined by both Primers 1 and 2 becomes predominant. It increases geometrically in amount by doubling with each cycle. After 30 cycles there are a billion copies of the reaction product but only 30 copies of the runoff products

FIG 2—Schematic illustration of some of the options available to analyse mRNA by the reverse transcriptase polymerase chain reaction. Having synthesised single stranded cDNA a variety of products can be generated by selecting pairs of the primer types A to F. As illustrated for primers A and C, linker sequences may be included at the 5' ends of any of these primers to further facilitate cloning of the resulting reaction products

fold excess of somewhat similar DNA fragments is even harder. The practical consequence has previously been that prolonged autoradiographic techniques were required. Days or weeks would be needed for each experiment. By simply amplifying the fragment of interest it can be characterised in agarose gels, usually by visual inspection, in a few hours.

MESSENGER RNA

Another example of the reaction's impact would be the use of the polymerase chain reaction to overcome some of the many problems inherent in analysing messenger RNA (mRNA) by classic techniques. These molecules, the ultimate source of information about what is going on at a specific time in a particular cell, are extremely labile chemically (for example, to traces of alkaline detergent in less than scrupulously clean glassware) and enzymatically (to the ubiquitous ribonuclease). A typical RNA preparation from a tissue sample or cell culture will contain around 2% of mRNA or less, with 98% ribosomal RNA (rRNA) and transfer RNA (tRNA). Though selecting for the polyadenylated mRNA on oligo(dT) affinity columns is possible, it is notoriously difficult even for the nimble fingered. Handling the minute amounts of material required remains extraordinarily difficult. Synthesis and subsequent cloning of copy DNA (cDNA) often yielded unsatisfactory clone banks with short inserts and heavy ribosomal DNA (rDNA) contamination for these purely technical reasons.

The situation is transformed by the use of the RNA polymerase chain reaction (fig 2). Oligo(dT) itself can be used to prime first strand synthesis of cDNA. Commercial kits are available and even thermostable "reverse transcriptases" have been introduced. Double stranded cDNA is obtained by a number of methods including using a second oligonucleotide⁶ or degen-

erate mixture specific for a target mRNA, or else total double stranded cDNA is produced by "tailing" the first strand and using the complementary homo(oligonucleotide) as the second primer.⁷ This has become known as "anchor polymerase chain reaction." After multiple cycles of amplification contaminating RNA species are no longer a problem and there is plenty of material to clone, sequence, or otherwise analyse, all within a few hours and with minimal manual intervention.

Incorporating the reaction into routine molecular genetics

ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

Analysis of restriction fragment length polymorphisms, previously a stock-in-trade of molecular genetics, has been revolutionised by the polymerase chain reaction. We have discussed the inherent difficulty of Southern blotting above. For restriction fragment length polymorphisms analysis it is essential, in particular, to generate DNA of sufficient quality to undergo digestion to completion with restriction enzymes. This is not always easy. Failure to cut sample DNA may lead to diagnostic errors with clinical material. Amplifying the target DNA means that obtaining high quality DNA should never be a problem, at least in principle. Control amplifications on samples to show that a constant restriction enzyme site can be cut strengthen confidence in the analysis. Furthermore, the presence or absence of polymorphisms can be assessed immediately by agarose gel electrophoresis and by inspection of ethidium bromide stained products under ultraviolet light.

The reaction has in fact permitted dramatic advances in the use of genetic polymorphism in linkage analysis. As well as classic dimorphic restriction fragment length polymorphisms, which are not particularly informative, several classes of repeat sequences have now been characterised in DNA which are highly polymorphic as a consequence of wide variation in repeat copy number.⁸ This is easily assessed by the polymerase chain reaction using sequences that flank the microsatellite or minisatellite repeat—the size of the product reflects the number of repeat units. The procedure has revolutionised human genetics by massively increasing the speed and power of pedigree analysis. Of importance to the clinician, certain inherited diseases (myotonic dystrophy, the fragile X syndrome, Kennedy disease) seem to be the result of spontaneous increases in the copy number of trinucleotide microsatellite repeats.⁹ Again, assays for this based on the reaction already permit pre-symptomatic or antenatal diagnosis.

DNA SEQUENCING

DNA sequencing, another fundamental molecular genetic technique and increasingly a vital component for identifying molecular pathology in patients, has also become reliant on the reaction. Traditionally, single stranded DNA for sequencing was obtained by cloning into the M13 bacteriophage system and subsequently purifying phage DNA. Given a reasonable amount of DNA template, sequencing was achieved by extending oligonucleotide primers in the presence of dideoxynucleoside triphosphate chain terminators. Amplification by the reaction solves the DNA yield problem without cloning. The material may be either sequenced double stranded or rendered single stranded by asymmetrical polymerase chain reaction (using very much less of one of the two primers),¹⁰ selective enzymatic digestion of one of the strands, or by selective capture of one of the strands by labelling one oligonucleotide with biotin and immobilising the

product on streptavidin coated magnetic beads. So called "cycle sequencing" can increase the sensitivity of the process. In many circumstances the use of high temperatures for the thermostable DNA polymerase reaction is advantageous in sequencing because secondary structure in the template strand is eliminated.

CONTAMINATION

There can be problems in controlling this powerful technique. As the polymerase chain reaction is able to amplify even a single molecule, contamination by any previously amplified DNA would be catastrophic. Many precautions have been devised to avoid contamination,¹¹ but scrupulous housekeeping is essential in any laboratory routinely undertaking the reaction. An extensive debate about the fidelity of copying DNA sequences by Taq polymerase has been published, but this is not usually an issue except in highly specialised applications, such as the study of variant clinical isolates of HIV.¹²

Some applications of the reaction

There is an enormous range of novel applications of the reaction, and the list of references contains several comprehensive reviews. Specific examples that have been particularly useful include:

Quantitative polymerase chain reaction, which permits measurement of the level of specific mRNAs in different cell populations.¹³ The amount of target product of the reaction is compared with the amount generated from a control amplification target in the same reaction. The technique has been most widely applied in the analysis of cytokine responses.

RACE-polymerase chain reaction. RACE stands for rapid amplification of cDNA ends. The reaction is performed between one primer designed on the basis of a given protein sequence and a second, which flanks the cloning site in a phage, or plasmid vector. The substrate is a total cDNA library. The product of the reaction is derived from the target mRNA, and the technique is particularly useful for identifying 5'-terminal cDNA sequences.

Alu-polymerase chain reaction is enormously valuable.¹⁴ Reaction primers match sequences in the ubiquitous interspersed repetitive sequences, of which about a million copies are scattered throughout the human genome. Amplification yields reaction products derived from the regions between Alu sequences. As the Alu repeats are relatively unique to humans, the human component from sources such as somatic cell hybrids in rodent cell backgrounds, or human DNA cloned in cosmids or yeast artificial chromosomes (YACs), can be isolated.

Inverse polymerase chain reaction permits amplification of DNA in which the sequence of only one end of the

fragment is known. This permits effective chromosome walking.^{15,16}

Vectorette polymerase chain reaction also permits amplification of regions of DNA of unknown sequence that flank known sequences. The approach entails restriction digestion of the DNA sample then ligation of specially designed "vectorette" linkers. The amplification is performed between the known sequence and the vectorette sequence. This method has proved generally useful in chromosome walking exercises and is particularly convenient for isolating the ends of yeast artificial chromosome clones for rapid physical genetic mapping and ordering of overlapping YACs to assemble so called "contigs".¹⁷

Specific applications of the reaction in molecular medicine

There are three distinct types of clinical challenge for which the polymerase chain reaction is indispensable: (1) Detecting vanishingly small amounts of DNA so as not to miss even the most cryptic infection and permit analysis of single cells or single sperm and of partially degraded samples. (2) Identifying the specific new mutation in a particular gene that causes a given inherited disease in a patient. This is necessary to gain an understanding of the molecular basis of the resulting disease and also to allow accurate family studies for genetic counselling. (3) Analysis to detect known mutations that always cause a particular inherited disease or polymorphism (for example, sickle cell anaemia, or the cystic fibrosis F508 deletion).

Detecting vanishingly small quantities of nucleic acid has been achieved in the diagnosis of HIV infection and in such unusual applications as detecting measles virus RNA in brain biopsy specimens from patients with subacute sclerosing panencephalitis. The nested technique is often helpful, and eventually the products of polymerase chain reactions may even be analysed by Southern blotting to gain further absolute sensitivity. In these circumstances, the issue of sample contamination giving false positive results is usually as important as the risk of false negative results arising from "failure" of the reaction. The ability to detect negligible (a few molecules) DNA is also essential for preimplantation diagnosis in in vitro fertilisation clinics.¹⁸

CHARACTERISATION OF GENE MUTATIONS

A range of elegant techniques has been developed for characterising new genetic mutations—all the techniques rely on the generation by the polymerase chain reaction of large quantities of DNA fragments from normal and mutant alleles. The problem is to detect as little as a single base pair difference between, say, two 500 base pair fragments. This is often achieved by creating heteroduplexes between the two alleles and looking for modified properties in: (a) chemical cleavage reactions; (b) ribonuclease digestion (one fragment is RNA); (c) denaturing gradient gel electrophoresis; (d) single strand conformational polymorphism; (e) reaction with carbodiimide; or (f) automated total sequencing of both fragments.

A smaller number of techniques based on the polymerase chain reaction is available for the routine detection of known mutations. If the mutation creates or destroys a restriction site then this can be simply examined in the products of the reaction. The disadvantage is that two distinct operations (polymerase chain reaction and restriction cleavage) are required. Detecting mutations by hybridising products of the reaction with allele specific oligonucleotides is possible, although again the need for two distinct procedures is a drawback.

Uses of the reaction in molecular genetics

- Analysis of restriction fragment length polymorphisms
- Analysis of messenger RNA
- Amplification of fragments for identification by Southern blotting
- Assessment of genetic polymorphism in linkage analysis
- DNA sequencing
- Preparation of elusive DNA fragments for cloning

The concept of an allele specific polymerase chain reaction, usually called "ARMS" (the amplification refractory mutation system), is rather more convenient and general.¹⁹ Here one of the pair of reaction primers is deliberately designed so that its 3' terminal residue lies precisely at the point mutation site. Two experiments are run in parallel: in one the allele specific primer matches the normal sequence, in the other it matches the mutant sequence. A product is obtained from the reaction only when 3' residues form base pairs and prime synthesis correctly. By comparing the products in the "normal" and "mutant" reactions, normal and mutant homozygotes as well as heterozygote carriers are easily identified. Many genetic diseases are now routinely tested for in this way.

Furthermore, several pairs of the reaction primers can be mixed in the same tube and allowed to "analyse" different point mutations at the same time (all the different products of the polymerase chain reaction are deliberately designed to be different sizes). By this "multiplex" approach, for example, all of the four (or more) common mutations causing cystic fibrosis can be looked for simultaneously in a single sample.²⁰ Several similar assays based on thermostable DNA ligases rather than polymerase are also being developed. A similar type of multiplex polymerase chain reaction analysis aimed at detecting DNA deletions has revolutionised antenatal diagnosis of Duchenne type muscular dystrophy. Another clinical advantage of these exquisitely sensitive tests has been that sufficient DNA can be obtained from buccal washings, dried blood on Guthrie phenylketonuria test cards, or tiny chorionic villus biopsy specimens.

APPLICATIONS

As the technique has developed so the range of applications in clinical practice has expanded. HLA molecular subtyping by polymerase chain reaction is straightforward, and clinicians are now attempting to develop tests that predict people at risk of developing insulin dependent diabetes mellitus on that basis.²¹ Mutations that appear to predispose to myocardial infarction (in the angiotensin converting enzyme (ACE) gene)²² and hypertension (in the angiotensinogen gene)²³ have been identified recently. Population screening programmes may be worth while. Both predispositions may be suppressible by treatment with angiotensin converting enzyme inhibitors. Various mutations in both oncogenes and tumour suppressor genes have been implicated heavily in the development of cancer. Assessment of the extent of such mutation in a given tumour may permit assessment of prognosis or predict whether distant metastasis has already occurred.

Future clinical possibilities

This raft of techniques can be applied to most specialties. Here I will limit discussion to two disciplines in which there is great activity.

ANTENATAL DIAGNOSIS

In antenatal diagnosis much effort is directed towards methods of analysing those few fetal cells found circulating in a pregnant woman's peripheral blood. By this approach invasive fetal sampling techniques may be avoided entirely or limited to those cases where confirmation of a positive diagnosis is desirable.

The estimate is that less than 10 000 fetal nucleated erythrocytes are present in 20 ml of maternal blood. Fluorescence activated cell sorting with anti-CD71, anti-CD36, or anti-glycophorin A, or a combination of these three monoclonal antibodies, permits enrichment towards 90% pure fetal nucleated erythrocytes. Trisomies can be detected direct by fluorescence in situ

Present uses of the reaction in medicine

- Detection of vanishingly small amounts of nucleic acid—for example, in HIV infection
- Identification of new genetic mutations
- Routine detection of known mutations—for example, Duchenne type muscular dystrophy, cystic fibrosis. Assays based on the reaction allow presymptomatic or antenatal diagnosis of diseases due to microsatellite instability—such as myotonic dystrophy, the fragile X syndrome, and Kennedy disease
- Detection of mutations thought to predispose to disease—for example, myocardial infarction and cancer
- Detection of mutations in malignant tumours to assess prognosis
- HLA subtyping

hybridisation. It seems to be only a matter of time before sufficient purification is achieved routinely to permit the full range of diagnosis based on the polymerase chain reaction as above. Indeed, this whole topic was resurrected some three years ago by the demonstration of Y chromosome specific product obtained by the technique from pregnant women with male fetuses.²⁴ This essentially confirmed that fetal tissue was in fact present in the maternal circulation.

DETECTION OF CANCER

Early detection of cancers is widely regarded as clinically important in that early treatment usually improves prognosis, and curative surgery may even be possible—for example, in colorectal cancer. The technique will have a major impact in this subject. It is already clear that mutations in p53 genes indicative of developing bladder cancer can be detected by analysis of shed cells present in patients' urine samples.²⁵ Even more remarkably, premalignant changes in the gastrointestinal tract can be detected by testing faeces using the polymerase chain reaction. Enough premalignant cells are present in the bulk of stool to permit the analysis of tumour suppressor gene mutations by this technique. Patients at high risk can thus be selected for colonoscopy. Whether this approach offers advantages over faecal occult blood testing remains to be established.^{26,27} The fact that gastric *Helicobacter pylori* infection has been assessed from stool samples by this technique suggests that gastric malignancy may also eventually prove detectable in this way. Detection of potentially metastatic cells in the circulation of patients with newly diagnosed primary tumours (for example, melanoma) is particularly intriguing.²⁸ The impending characterisation of a melanoma related tumour suppressor gene from human chromosome 9 should make this type of approach even more useful.²⁹ The clinical usefulness of the polymerase chain reaction thus seems to be limited only by the power of our imagination in identifying specific targets.

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Letter from Albania

A country in transition

Hans Veeken

"That will get stolen," the minister of health exclaims when I show him the picture of the pressure cooker that is meant to be used as a steriliser in the dispensaries. He immediately realises the impact of his remark and adds reassuringly: "We will take measures to prevent that, of course."

Working as a medical coordinator for Médecins Sans Frontières I had the privilege to work in Albania during the period of transition since communism has been discarded. I arrived at Christmas 1991. There was snow and frost, and the minister received me in his fur coat because there was no heating. During the following months we started to distribute basic medical material to dispensaries, health centres, and hospitals in a few districts. I travelled extensively throughout the country for six months to prepare for a nationwide extension of the programme, which was funded by the European Community.

Basics to start with

The director of the district hospital in Corovoda shows me his hospital with enthusiasm. "Have a cigarette," he says, and as if to persuade me he lights one himself in the corridor. The ash will be scattered on the floor throughout the hospital. "Can't you provide us with a new x ray machine, incubators, analysers, and an ultrasound machine? These are things that every hospital needs, don't you agree?" The director is visibly disappointed when I explain that we will start with the basics and that we will not spend the whole budget on a new x ray machine.

The building is decrepit, the doors do not shut, the windows have no glass, some taps leak continuously, the staff are too embarrassed to show me the lavatories. The guided tour takes the form of a procession; all doctors are interested to speak to a foreigner. Despite their isolation the doctors often speak foreign languages. During the visit I do not get the impression that I'm keeping any of them from their work. The

reason is simple: there are hardly any patients. "Are the Albanians that healthy?" I ask innocently. He grabs me amicably by the shoulder, pinches me firmly, and explains that since the Chinese left no new equipment has been purchased. The patients realise this and they do not expect any effective help anymore: medicines, sutures, needles are all scarce or absent. Furthermore, it is so cold during this time of the year that, without heating, patients do not want to be admitted.

The way that doctors and other staff associate with each other is much less formal than we are used to in Western countries. The level of their salaries is equally striking: doctors earn \$14 a month and nurses \$10. At an average expenditure of \$17 per head, the budget of the Ministry of Health is scarcely sufficient to pay the salaries, let alone to purchase any new equipment. The scarcity is beyond words; even the most basic materials such as gauzes, needles, and syringes cannot be found. The hardware, such as x ray, theatre, and laboratory equipment, is still of Chinese or even Russian origin, with some items being over 40 years old. Autoclaves are functioning but neither the pressure gauge, thermometer, nor the timer are working. The sterilising procedure is guided by intuition alone, the high prevalence of hepatitis B proves that this is not the best method. In the corridor I'm addressed by another doctor. "Do we get a sphygmomanometer? How can we work seriously without one?" A sphygmomanometer, a thermometer, scales—all are rarities in the hospital. The surgeon presses his stethoscope in my hands: "What is your opinion of this patient?" he asks. The stethoscope keeps silent. I adjust the earplugs, with no result. Helplessly I look up from the patient. The doctors laugh and the surgeon says: "Yes we do know how to examine our patients, even without proper equipment," as if to convince me of their capabilities. I examine the stethoscope instead of the patient and notice that the membrane is missing.

The statistics suggest that people's health is not too

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