Lewis Cameron Undergraduate Prize.

THE CONTROL OF VIRUS INFECTIONS.

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Historical Aspects.

Since my childhood, I have had the patriotic notion that everything worthwhile was invented by the British, and anything invented before the British were capable must invariably have come from the Chinese. However many and glaring may be the exceptions to this biased rule, the first method of artificial protection against viruses was invented by the Chinese, and later modified by an Englishman, Edward Jenner, into a safe, effective and easily administrable form.

The Chinese claim to have inoculated against smallpox since the eleventh century, using swabs from a virulent case which were inserted into the nostril. In 1721, the wife of the British Ambassador in Constantinople introduced vaccination into England. However, this prophylactic measure involved the use of the smallpox virus itself, and was not without considerable risk. Jenner made the next most significant step by using the relatively harmless virus of cowpox to vaccinate his patient, a boy James Phipps, who, eight weeks later, proved to be immune to a challenging vaccination with smallpox virus. Even this step was only taken twenty years of deliberation after hearing the chance remark of a dairymaid - "I can't take smallpox, for I have already had cowpox" - for smallpox is a terrible disease.

For this same reason, the search for methods to control the disease has been a very active one, and has ultimately led to an almost complete eradication from this country. The procedure of vaccination is not without slight risk of generalised vaccinia and post-vaccinial encephalitis, but surely this does not warrent the demand, made by certain groups, that the procedure should be abolished. One of the most important purposes of history is to inform enquiring minds why certain aspects of society are as they are to-day, and surely a study of the history of vaccination must sober the most ardent abolitionists.
Jenner's hesitation can be well understood if it is considered that he had no idea of the underlying principles of immunology and virology involved. However, even if their causes were not known, the effects of many virus diseases were known to our forefathers, and preventative measures arising from empirical observations resulted in attempts at control. Most important among these were isolation and quarantine. The latter term derived from the French 'quarante', or forty, meant the forty day isolation of passengers and crew from ships which had sailed from ports where diseases were prominent. It was first introduced during the Black Death in 1377. This method cannot be enforced to-day because of the frequency and economic necessity for travel, but all cases of smallpox occurring in Britain to-day are 'imported' by travellers entering the country.

The only really satisfactory way to influence the pattern of virus diseases is to increase the resistance of the individual artificially. The patient's natural resistance must be boosted, or be aided by chemo-therapeutic agents.

Theoretical Considerations.

Viruses were separated as an entity in their own right in 1892, when Ivanowski found that the agents responsible for the tobacco mosaic disease would pass a filter that held back the smallest living organisms then known. Perhaps the paradox is that virus diseases were the first to have protective measures developed against them artificially, but were the last to be explained. This was because, although viruses behave immunologically in a similar manner to bacteria, their pathogenicity is more fundamental, and their size much smaller. 'Fundamental' is indeed no hyperbole, for with the development of the electron microscope and delicate techniques of ultracentrifugation and tracer marking, virology has drawn the research worker into the core of the primary problem of life itself, that of replication.
By the use of the techniques mentioned above, the virus has been shown to consist essentially of nucleic acid, surrounded by a layer of protein. By parallel research, the genetic material of all cells has been shown to consist of deoxyribonucleic acid, contained in the nucleus. Certain investigators, notably Crick and Watson, have put forward the theory, reasonably substantiated by evidence obtained from X-ray diffraction experiments and others, that information constituting instructions for the synthesis of the cell's proteins is embodied in a double-chain molecular helix of deoxyribonucleic acid (D.N.A.). The coding takes place in the arrangement, principally, of four different bases. Adenine, Thymine, Cytosine and Guanine, which project from the edge of the helix. Calculation shows that a sequence of three of these bases is sufficient to specify any one of the twenty available amino-acids for inclusion into a protein molecule.

Without going into details, it has been postulated that from this central code recorded on D.N.A. in the nucleus, templates of ribonucleic acid (R.N.A.) are 'cast', called 'messenger R.N.A.'. These proceed into the cytoplasm, where microsomal particles called ribosomes pass along them 'reading' the sequence recorded and incorporating each 'activated' amino-acid from the cytoplasm in the appropriate position in the chain of amino acids constituting the protein molecule. Thus, from the nuclear 'key' of D.N.A. is dictated the pattern of cellular protein synthesis.

The reason that viruses, with the exception of the Psittacosis/Lymphogranuloma Venereum group, do not respond to recognised chemotherapy is because they do not reproduce in the classical manner of binary fission, nor do they show mating of male and female elements according to Mendelian law. Also, they require an intracellular habitat before multiplication occurs, and possess no individual metabolism. From this information, and from the fact that they consist almost
entirely of the same material as the genetic system of a cell, it has been proposed, with some reason, that viruses are in fact roving particles of genetic material. These enter cells, and perhaps by competing with the nuclear material or the messenger R.N.A., form a rogue template from which the ribosomes of the invaded cell synthesise not cellular protein, but viral nucleoprotein. In this way, the virus utilises the metabolism of the host cell to reproduce itself, carrying the art of the cuckoo one step further - the host actually reproduces the young as well as hatching them. This might appear to render the concept of virus chemotherapy an impossibility, as in order to stop viral reproduction, the metabolism of the host's cells must be interfered with. This, if unselective, would obviously harm the patient. However, the infected cell may be more penetrable than normal cells, and also it seems likely that once a virus has infected a cell, the nucleic acid producing mechanism of the infected cell is qualitatively and quantitatively different from that of a normal cell, and is therefore subject to inhibition by therapeutic agents which may be more or less selective. In such a cell, it may be difficult to stop synthesis of viral nucleic acid before serious damage is done.

Relying on these properties to differentiate the virus infected cell from the normal cell, two theoretical possibilities present themselves. Firstly, a chemical agent might be capable of interfering with the utilisation or synthesis of a natural metabolite. An example of this could be the interference with incorporation of amino acids into proteins by an action on the ribosomes. Secondly, an analogue of a metabolic intermediary, such as a purine, pyrimidine or amino acid could be introduced into the cell in the hope that by competitive inhibition it might be incorporated into the end product, the virus, rendering it atypical and ineffective. In this respect, it is interesting to note the findings of Martin and Work (17) that the appearance of viral R.N.A. precedes the appearance of viral protein by about 1 - 1½ hours, the latter co-inciding almost
immediately with the appearance of formed virus. Thus, amino acid analogues, such as D-1louro-phenylanaline are unlikely to be of much value by themselves due to the short period before the newly synthesised protein is incorporated with the nucleic acid as complete virus.

However, this whole subject is very complex and by no means elucidated. Most effective antiviral agents, it must be admitted, have been discovered more by chance than by good management.

Specific drugs.

The psittacosis/ornithosis group of viruses and the lymphogranuloma venereum virus can be effectively treated by tetracyclines. Early studies of the chemotherapy of this group were mainly interesting in regard to the possibility of developing a carrier state through incomplete eradication of the virus. The relevance of this to the dynamics of virus infection is considerable. However, since 1950, efficient eradication is possible if tetracyclines are properly used. In chick embryos, chlortetracycline is five times as effective as chloramphenicol. With what has been said before in mind, this may seem rather surprising. In fact, the reason for the effectiveness of standard chemotherapy has not been entirely elucidated, but this group of viruses is not typical in its mode of reproduction. After invasion of a cell, the elementary body disappears for a period of 8-30 hours, depending on the strain. Infection is manifest by the reappearance of round 'initial bodies', about 600 μ in diameter. These bodies divide repeatedly into smaller and more numerous bodies until the final elementary body stage is reached. It was suggested by Bedson, after a lifetime of experience, that the division in fact took place by binary fission, and this has been supported by the observations of Swain and Gaylard. In any case, the action of tetracyclines in vitro and in vivo is to suppress division of the initial bodies, and one assumption is that these compounds interfere with
some metabolic process essential for reproduction. With an obviously more complicated cycle of reproduction, it is reasonable to presume that this virus possesses more metabolic machinery than the smaller viruses, and is thus more susceptible to standard chemotherapeutic agents. A similar virus is that of trachoma, which is also susceptible to tetracyclines. Sulphanmethoxypyridazine is more effective, as it has a prolonged action. Another virus sensitive to tetracyclines is the agent responsible for 'virus' pneumonia. Its precise nature has not yet been determined, as it is not visible by the light microscope like the other antibiotic-sensitive viruses. One suggestion is that it is a mycoplasma, but more than one agent may be responsible.

When a knowledge of virology had developed sufficiently for cultivation techniques to be widely used, numerous compounds were tested by a multitude of workers for anti-viral activity. In 1950, Hamre et al.(16) tested p-aminobenzaldehyde 3-thiosemicarbazone. This compound was chosen as the most soluble of a series of thiosemicarbazone derivatives found to be effective against tuberculosis, and was active against vaccinia virus infection in fertile hen eggs. The same substance was found by her to delay the death of mice intranasally infected with vaccinia virus. Thompson and his colleagues (19) confirmed this work, showing that isatin, and 5-nitro 2-thenaldehyde thiosemicarbazones protected the majority of mice intracerebrally injected with William-sport virus (a member of the variola-vaccinia group), and later followed this with similar experiments using the vaccinia virus. From these latter experiments they concluded that thiosemicarbazones containing benzene, thiophene, pyridine, quinoline or isatin group protected mice against vaccinia virus.

The method of action of the thiosemicarbazones is not known clearly at present, but minor substitutions to the molecule can abolish its effect. In this respect, Thompson postulated that the presence of a \( -\text{N} - \text{NH} - \text{CS} - \text{NH}_2 \) group and a cyclic of heterocyclic
component were compatible with high antiviral activity. 

More recently, Bauer (5) has done some detailed work on this aspect using the ectromelia virus. Whereas isatin \( \beta \)-thiosemicarbazone and certain of its derivatives have been shown to possess a high degree of activity in animals infected with vaccinia, rabbitpox, alastrim, variola major and white cowpox. isatin \( \beta \)-thiosemicarbazone itself has no action against ectromelia virus. By an ingenious series of substitutions, the activity of the compound against this virus could be altered. and comparisons drawn using its activity against vaccinia virus as a control. It might be of interest to consider these briefly as some indication of the possibilities open to workers in this field in the future.

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\text{(a) Isatin } \beta \text{-thiosemicarbazone}
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Intracerebrally inoculated mice were used, and the effect observed by measurement of the virus titre in ground up brain.

If the two hydrogen atoms of the terminal amino group of the side chain were replaced (fig. a) by alkyl groups \((R_1, R_2)\) in fig. b), \(N\)-methyl \(4':4\)-dimethyl thiosemicarbazone resulted. This had the same dose response curve against ectromelia as isatin \( \beta \)-thiosemi- carbazone against vaccinia, but activity against vaccinia and the other viruses was abolished. Furthermore, the substitution of only one of the hydrogen atoms left the molecule with no antiviral activity whatsoever. Increasing the chain length of the substituted alkyl group progressively diminished the activity against ectromelia. Further findings were that alkylation at the 1-position reduces antiviral activity against ectromelia, in contrast to the increase in activity against vaccinia. Also, substitu-
to a greater extent than against ectromelia.

Apart from the hope these findings may offer to research pharmacologists of the future, two important things emerge. Firstly, the protection against death afforded by the thiosemicarbazones is due to a virostatic effect, as shown by the reduced virus titre (after 4 days, only $10^{-3}$ of the amount of virus in the untreated control remained). Secondly, they suggest that their effect must be on the virus particle itself, and not on a system in a host cell, as in each case, the host cell system is identical. The variation in effect must thus be a reflection of differences in the constitution of the viruses at the molecular level.

Obviously the next step, which was taken by Bauer and colleagues (4), was to test the efficacy of this drug against smallpox in vivo. This was done in a controlled trial using contacts of patients admitted to a Madras hospital, randomly assorted into control and treated groups. Suffice it to say that only 3 out of the 1,101 treated cases contracted smallpox, whereas 78 (with 12 deaths) cases occurred among the 1,126 untreated controls. The drug was administered by mouth, and no side effects worse than nausea and vomiting were observed. It was concluded that the drug used (N-methylisatin $\beta$-thiosemicarbazone) reduced the incidence regardless of vaccination status, over and above the possibility of the disease having been contracted from an earlier source. As a method of treatment, it is conclusively superior to re-vaccination and antivaccinial $\gamma$-globulin.

However, the same drug seems to be ineffective against vaccinia necrosum developing after vaccination.BLEWETT and KeR (3) accredited the drug with, at the most, producing a slight drying up of lesions and perhaps prevention of further lesions, whilst Connolly et al. (20), using the drug applied as a cream direct to the lesions could see no benefit from it. However, both cases were babies with hypogammaglobulinaemia who failed to respond to anti-vaccinial $\gamma$-globulin in large amounts and interferon, and were in a late stage when brought to treatment.
As a potentially toxic drug, its side effects seem to be minimal when taken by mouth, although Connolly reports the development of a blotchy erythematous rash on the hands, feet, back and abdomen, together with the mother's claim that the baby's hair was beginning to fall out. The rash faded when the drug was stopped. Hitzig and Willi reported a morbilliform rash occurring in a similar case treated with γ-globulin only.

In the thiosemicarbazones, therefore, it appears we have the makings of an effective antiviral chemotherapeutic agent. However, it is a relatively toxic drug and seems to produce a satisfactory result only when applied in the early stages of an infection. In this latter respect, it is an improvement on existing therapy, but as yet it is a pale shadow of its antibacterial counterparts.

Whilst these drugs were discovered by chance, the practical application of the theoretical concepts we have discussed has not been without some success. The use of nucleoside analogues, especially of halogenated ones, as potential competitive inhibitors of the incorporation of the naturally occurring nucleosides cytidine, uridine, thymidine, adenosine and guanosine was extensively investigated by Salzman, Smith and Tamm in 1960. In 1961, Hermann, using a plaque inhibition test (22), showed that 5-iodo 2-deoxy uridine (I.D.U.) inhibited growth of vaccinia and herpes viruses (D.N.A. containing viruses). A similarly effective substance was 5-bromo 2-deoxyuridine, but neither of these had any effect on two R.N.A. containing viruses. This was interesting, as in D.N.A. thymidine is a primary constituent. A comparison of chemical structures illustrates that I.D.U. resembles thymidine (figs. c and d), except that the 5-methyl group is replaced by an iodine atom. As such, the I.D.U. is in a position to competitively inhibit the uptake of thymidine into D.N.A. However, R.N.A. contains little or no thymidine, and is composed of a much higher concentration of uridine - which has no side group at the 5-position (fig. e).
Besides competitive inhibition of thymidine uptake, I.D.U. may be itself incorporated into an aberrant DNA molecule, which presumably cannot be used to form infective virus particles. If its action is as suggested, this drug should be considered as a general antimetabolite, relying on the properties of the virus infected cell (namely increased permeability of the cell membrane and the qualitative and quantitative differences in nucleic acid synthesis) to make these cells more susceptible than normal to its effects. In any case, systemic application is impossible due to the low solubility of the drug in water. It has found practical application, nevertheless, in the treatment of corneal ulcers, especially those caused by herpes simplex. The cornea is an avascular organ, and is similar to a tissue culture in many respects. This fact was utilised by Kaufman and his colleagues, who carried out two series of experiments on the eyes of albino rabbits. The central cornea was traumatised with circular trephines in both cases. In the first series of experiments (15), herpes simplex was instilled into the damaged area. The rabbits were then randomly assigned to saline treated control eyes, and eyes treated with I.D.U. drops every two hours round the clock. In all cases, even when the drug was first administered 72 hours after infection, healing promptly followed administration, although after stopping treatment, one out of four eyes contained culturable virus, and 16 out of 43 had recurrences. The next step was to use a strain of virus causing a deep, or stromal infection. Under these circumstances, I.D.U. only cured 7 out of 17 eyes under similar conditions to the above.

A later experiment (6) was to substitute vaccinia virus for the herpes simplex where results were essentially similar. However, it must be emphasised...
that since the effect is one of competitive inhibition as far as nucleoside uptake is concerned, the need for maintained concentrations of the drug is vital. If applied adequately, this drug is capable of exerting its effect even when the condition is advanced, and thus approaches more closely the ideal chemotherapeutic agent. It might be said that as this is a powerful antimetabolite administered locally, it is hardly to be compared with the systemically administrable anti-viral agent eagerly sought after to put anti-viral chemotherapy on a par with anti-bacterial agents.

Also, a realisation that this drug was developed for its possible cytotoxic and therapeutic efficacy in the treatment of cancer (Prusoff, 1960) (23, 24) suggests the possibility that it will interfere with normal cells and have harmful effects. However, Kaufman could find no evidence of deleterious effects on the corneal cells attributable to I,D,U. This supports the likelihood of a selective method of action, even if the corneal epithelium was removed completely from the eye of a non-infected rabbit being treated with I,D,U., healing of the cornea did not seem to be in any way inhibited. The clarity of the stroma was maintained and no corneal oedema occurred. Even after a month's administration, no abnormalities were seen.

Following this experimental work, a clinical trial was performed, as the drug opened up a new field of treatment for the serious and potentially blinding condition of dendritic corneal ulceration (7). Treatment consisted of a drop of 0.1% solution in the affected eye hourly, day and night for 3 - 11 days, depending on the effect. Cases of primary corneal ulceration without stromal involvement were found to heal in 3 - 4 days, whilst in those with stromal involvement healing was completed in 4 - 6 days. However, in four of the cases, punctate staining was noted after five or six days application. Secondary, non-dendritic ulceration and epithelial degeneration were also observed in two cases, one of which progressed to a large erosion, persisting for 20 days. These, together with the staining, were attributable in the investigator's
opinions to the drug, as the complications were rapidly reversible on withdrawal of therapy, and resembled the damaging effects produced by excess dosage of ultra-violet light. However, the dosage was twice that used by Kaufman, and clearly the optimum duration and frequency of treatment remain to be elucidated.

Several other antiviral substances have been tried, especially by Perkins (21) and Richtsal and al. (10) who found guanidine salts to have some action against polioviruses and some other enteroviruses. These are R.N.A. containing viruses, and as guanidine is a principle constituent of R.N.A., it is reasonable to suppose that it has its effect in a manner similar to I.D.U. Guanidine salts cannot be used in man because of severe toxicity, but their ability to inhibit the cytopathic effect of poliovirus in cell culture has been confirmed in vivo against monkeys infected with polio, and indicates the value of tissue culture techniques in the isolation of potential antiviral agents.

Another chemotherapeutic agent which has been tried against acute respiratory illness and measles in man is 'Virugon' (N,N'-anhydrobis( hydroxymethyl biguanide hydrochloride)). However, the treated groups have shown no superiority over control patients and it must be assumed that this drug is useless (8, 9).

The problems of chemotherapy of virus infections has been dealt with here in some length, not because chemotherapy is the principle weapon in the armoury of instruments effective in controlling virus infections, but rather because it has the unrealised potential or becoming so. Existing methods are clumsy, though effective, but offer no solace to the patient already exhibiting symptoms and signs of infection. While this state exists, the problem of developing antiviral agents in line with antibacterial agents will continue to challenge top research workers.

Here a small trickle has been shown, but this gives hope that once the dam is breached, this will turn into a flood of new agents.
Concerning the Natural Defences of the Body.

Comparatively speaking, very few virus infections are fatal or even serious. This is ample demonstration of the fact that efficient methods for controlling virus infection must exist within the body. As was said in the introductory part of this essay, the only satisfactory way to influence the course of virus diseases is to boost the natural resistance of the host, or aid it with chemotherapeutic agents. Having considered the latter in some detail, we must now turn out attention to the former.

Methods for boosting the natural resistance of the host were dependant on the unearthing, by prolonged research, of the natural processes involved in the host's reaction to an invasive micro-organism. It is impossible to attempt to aid a system one knows nothing about except by the shrewd exploration of chance, as with Edward Jenner. However, following his work, the idea that a mild attack of disease could confer protection against subsequent attacks was established, and formed the background for Pasteur's work nearly a century later. He was interested in the search for attenuated strains of viruses and bacteria suitable for use in vaccines, and had considerable success, notably in protection against rabies. However, it was not until von Behring and Kikasato showed in 1890 the presence of antidotes in the patient's serum which afforded protection against tetanus toxin, that much idea of the mechanisms underlying this development of immunity could be realised. The concept of antibody-antigen reactions was developed from this work as a result of much research effort, integrated with the rapidly advancing science of bacteriology. According to this well proven concept, the body forms an antibody which combines with, and neutralises the effect of an antigen. The antigen can be any substance foreign to the body, such as a virus particle. The specificity of the antibody is dependant on the structural morphology of the molecule of antigen. Thus any molecule which has an identical structure of the relevant portions concerned in antigenicity to that of a virulent virus molecule will be able to induce the body to form antigens which...
will neutralise not only the molecule itself, but also the
virus. The phenomenon discovered by Jenner now becomes
clear. The relatively harmless cowpox virus was capable
of inducing the formation of antibodies identical to
those which neutralise smallpox virus. These antibodies
are referred to as numoral antibodies, and make up the
Y-globulin fraction of the blood.

That this is not the whole story as regards immunity is indicated by other observations.
Firstly, it has been noted that children with the inherited
condition of agammaglobulinaemia, and who are thus incapable
of possessing numoral antibodies, show a normal reaction,
disease course and following immunity to measles, chicken-
pox and mumps infections. This indicates that an immunol-
ogical process exists distinct from the numoral system.
Other anomalies have come to light in bacteriology, such
as the persistence of diseases like brucellosis 6–12
weeks after the development of a high numoral antibody
titre, and the possible recurrence of typhoid fever if
the disease is quickly terminated, by chemotherapy, after
antibodies have developed. The immunological process
responsible for these observations is thought to reside
in the cells themselves, and has been termed cellular
immunity.

The cowpox virus is not used today to
vaccinate against smallpox, but an attenuated form of
the variola virus, developed by repeated animal passage,
called vaccinia virus. It can be prepared, in a method
developed by Jenner, from the lesion forming on an
innoculated calf. The material obtained by scraping the
lesions is rendered bacteria-free by 1% phenol applied
for 48 hours at 22°C., followed by glycerol, added in
such an amount that the final product contains 40%
glycerol and 0.04% phenol. This is termed 'calf lymph',
and is usually freeze dried ready to be made up for
vaccination. The vaccination is carried out by introducing
the virus into the deep layers of the epidermis by
multiple pressures of the point of a sterile needle held
almost parallel to the skin surface. This is the accepted
method, but McClean (27) and Cross (28) have expressed
the opinion that a single scratch not more than 6 mm.
long gives a higher success rate. A red papule appears, which develops to a crusted vesicle, leaving humoral immunity for five to seven years. However for complete protection, re-vaccination every three years is recommended.

The effectiveness of this procedure is shown by a recent comparison of the incidence of smallpox in the ten American states with compulsory vaccination (6.6/100,000) to that in other states (51.3 - 115.2/100,000) (14).

Pasteur's method of immunisation against rabies is much the same in principle. The 'street' virus is changed to a 'fixed' virus by serial passage in rabbits, and then submitted to varying degrees of attenuation by drying. This vaccine is in fact of doubtful value, but a more recent preparation developed from an egg adapted strain has been shown to be antigenic in man. Veeraraghavan (1959) carried out a trial of the incidence of rabies in persons exposed to the bites of animals definitely shown to produce rabies in other susceptibles. He compared the occurrence in those vaccinated with those refusing vaccination, and this he found to be 3.35% against 49.2% respectively. The value of the vaccine is undisputable, but it does not abolish the incidence.

The partial solution lies in the concomitant administration of anti-rabies serum (i.e. serum containing anti-rabies antibodies), and as this reduces the incidence considerably, it has been adopted as international procedure (W.H.O. 1957).

The reason for mentioning these two procedures in detail is that they both embody the principle of using the living variant of the causal virus to induce antibody formation in the absence of the disease itself. The alternative to this is to use an inactivated form of the causal virus which still retains the morphological features necessary to induce antibody formation. The first vaccine developed against poliomyelitis was of this nature.
The culture of poliomyelitis virus by Wendt et al. (29), followed by the demonstration of a method of inactivation by a 1 : 4,000 solution of formaldehyde at pH 7 and 37°C. by Salk (1953), which left the virus with its antigenicity, paved the way to the development of a killed virus vaccine. This consists of all the three strains of poliovirus (trivalent), and is tested exhaustively in animals for bacterial sterility and lack of virulence. The Salk vaccine was extensively used in the control of polio. According to a W.H.O. Expert Committee, its effectiveness is probably 80 - 90%, and its effects may persist for three years or more. However, it must be administered by injection three to four times to ensure adequate antibody levels, with the concomitant difficulties in mass immunisation. The standardisation and production of potent vaccine is difficult, and the dosage critical. Also, it does not prevent the multiplication of the virus in the intestinal or respiratory tract, and thus does not hinder spread. This means it cannot eradicate the disease from the community and is ineffective in controlling epidemics once they are underway. Thus, while this is a safe and effective method of conferring individual protection, it is by no means ideal, and the development of a living virus vaccine was awaited with keen interest in the hope that it would offer an improvement. However this was by no means easy, and the credit for its ultimate development must go to Koprowski, and, especially, to Sabin. Not only were there difficulties in obtaining an avirulent strain by repeated animal passage, followed by testing for 'cerebral' and 'spinal' neurotropism (17) in monkeys, but the risk of using an attenuated strain is always that it may revert to type with return of virulence, especially if spread to another individual. Smallpox and yellow fever vaccines are carried by the vaccinated individual for a sharply limited period. The attenuated polio strains are designed to cause a mild local infection in the intestine, and they have been shown to persist in the human alimentary tract for many weeks. Of course, this is one of their values, as they can prevent the multiplication of a virulent strain in the intestine, thus preventing its spread in a community.
Also, they can themselves infiltrate the community, building up a generalised resistance to the virulent strain; but the possibilities of a return to virulence are high in consequence.

Oral polio vaccine is now in regular use, although in America, suspicion of vaccine induced poliomyelitis has occurred in one or two instances. It can be easily applied as two drops on a lump of sugar, but one of its disadvantages is that separate inoculation for all three strains is required, as one strain causes 'interference' with another in the intestinal cells.

Its effectiveness has been demonstrated in Russia, where massive campaigns to eradicate polio have been carried out. The abrupt interruption in the seasonal pattern of polio which resulted was hard to attribute to anything but the vaccine. Smorodinsev et al. (1960) reported on 1,700,000 children contacts. From the 75,000 who were vaccinated in this number, there were only three cases of paralytic polio in the subsequent ten months, as against 41 cases from the 77,600 unvaccinated children. This is a thirteen times reduction in incidence. They reported the safety to be about one paralytic case in 100,000 subjects, but, of course, this is difficult to assess (14).

A trial of oral vaccine on 800 subjects in Switzerland by Goffe and Schar (1961) showed that 98.9% had type I antibodies, 99.6% type II and 98.3% type III antibodies in their serum (30).

Various vaccines have been developed against influenza, but the weakness of these lies in the strain variation found in the viruses causing fresh epidemics. According to a M.R.C. trial of a vaccine developed against the Asian strain, a 2/3 reduction in attack rate, compared with a non-Asian vaccine, was produced. It is in fields of mass vaccination in an attempt to abolish a virus disease that immunisation has its potential fully realised. As smallpox now occurs only occasionally in this country, due to vaccination, so may we hope for the
abolition of polio. However, importation of cases continues to occur, and world wide programmes (under the supervision of W.H.O.) leading to complete eradication, are hopes of the future.

Perhaps the outstanding recent success in this field has been the programme of immunisation against yellow fever. Two strains of avirulent yellow fever virus, the 'French neurotropic' and the '17D', isolated after repeated animal and tissue culture passage, have been used, one injection conferring immunity for over six years. An effort was made to vaccinate the entire population of Columbia. From 600,000 people who were vaccinated in the endemic area, only one case occurred (5 days after vaccination), whilst in the 10% of the population who did not co-operate, 198 proved and 45 probable cases occurred in the same period. Following this, the French government has embarked on a programme of vaccinating every person in the endemic areas or their colonies every four years. Vaccination is the only method of control of jungle yellow fever, as it is impossible to eradicate the mosquitoes and monkeys from such impenetrable areas. Thus, in Africa, vaccination is of particular value. The mosquito concerned is Aedes aegypti, which is also the vector of dengue, and interference has been known to occur between the two viruses in the common vector.

Jenner was probably the first to record the modification of one virus infection by another unrelated one, when in 1804, he wrote "These herpetic affections which so frequently appear among the children of the poor, and which are evidently contagious, often prevent the vaccine from producing its correct action". Many years later, in 1935, Hoskins found that a neurotropic strain of yellow fever virus which caused a mild infection protected monkeys against the lethal viscerotropic strain. This protective effect was shown by Windlay and MacCallum in 1957 to be unrelated to antibody production, and was termed interference. Twenty years later, Isaacs and Lindenmann suggested an explanation when they found that a heat killed influenza virus caused a cell culture to produce a substance, which accumulated in the culture.
fluid. This fluid, when mixed with fresh cells, protected them from virus infection (41). The substance responsible was isolated, and found to be a protein of molecular weight 63,000; stable at pH 2.0 and non-toxic and non-antigenic - even in heterologous animals. They named the substance 'interferon', and had great hopes for its therapeutic application. However, seven years later no therapeutic benefit has been derived, though gradually, stimulated by research on interferon, a mass of interesting speculation surrounding the phenomenon of cellular immunity (referred to earlier) is growing up. To understand the relevance of this, it must be appreciated that humoral antibodies can only act on a virus particle as it travels from its point of entry to the body to the precincts of the host cell. Many viruses, especially those causing respiratory conditions, never pass into the circulation at all, but enter their host cells directly. However, the recovery of the patient demonstrates that some other protective mechanism must be in operation.

It has been shown that under certain conditions, a virus and culture cells can co-exist, the virus being propagated along with the cells, but not destroying them (Ho and Wadars). This constitutes a 'recovery' by the cells. In such a culture, high interferon levels are present. An example of this in man is the herpes simplex virus, whose presence is only indicated by periodic bouts of virulence.

Also relevant is the recent discovery that certain viruses are pathogenic to one species of cell only, but are capable of inhabiting other types of cell harmlessly for long periods. Such a virus is the poliovirus, which is highly pathogenic to nerve cells, but will survive and multiply in a variety of tissues in primates, including pharynx, intestine and lymph nodes.

Briefly, interferon is thought to be a substance produced by cells on response to the entry of foreign proteins (13) or nucleic acid to the cell.
Homologous R.N.A. applied to chick, mouse and rabbit cells respectively will not induce interferon formation, but the same preparations after treatment with nitrous acid will do so. The nitrous acid must alter the R.N.A. sufficiently to render it 'heterologous' (33). However, there is not a wide species difference in interferon, as might be expected from experience of hormone studies. Interferon from various species of cells had been found to inhibit development of vaccinial lesions in the skin of rabbits and monkeys. Although homologous interferon was the most effective, significant inhibition was produced by heterologous preparations (38). Another interesting discovery was promoted when Lwoff and Lwoff (1960) stated that a virus infection was influenced in vivo and in vitro by temperature. Isaacs seized on this, and proceeded to show the correspondence between the optimum temperature for growth of a virus and its sensitivity to interferon. The higher the temperature, the less the sensitivity.

Of a similar nature was the statement by Enders (1960) that an avirulent strain of measles virus produced more interferon from human amniotic cells than a virulent strain. This obviously ties up with the ability of cells and viruses to co-exist, as mentioned above. Perhaps the virulence of a virus is proportional to its ability to stimulate its host cells to produce interferon? In an analysis of lung specimens from 11 fatal cases of influenzal pneumonia (39) a complete absence of interferon was noted.

It must be realised that, as regards evolution, the most successful virus is the one which attains a state of co-existence with its host; as in any branch of parasitology. The death of the host is rarely beneficial to the virus. This state of affairs obviously favours the less virulent virus, such as those of common infections, especially of the respiratory and pharyngeal tracts, that thrive in civilised communities. Speculation along these lines has lead to the thought that perhaps interferon is a normal cell constituent,
implicated by chance in virus infections. Isaacs has suggested that it may normally be concerned with cellular homeostasis, having some restraining effect on aerobic respiration of cells by uncoupling oxidative phosphorylation. By a feedback mechanism, interferon produced by an increased metabolic activity, such as occurs on virus infection, would serve to slow down the metabolism. Evidence in favour of this is that the changes produced by interferon closely resemble those produced by agents known to be capable of uncoupling oxidative phosphorylation, such as dinitrophenol, but without the attendant toxicity. Also, interferon has no effects in cancer cells. As these cells have been shown by Warburg to rely on anaerobic respiration, this would be expected if the hypothesis is true. Similar cells are those of embryonic tissue, and these also are unaffected by interferon, on assault by a virus. Isaacs has suggested that the deformities produced in the foetus of a mother contracting rubella may be due to this fact. However, he could not demonstrate any decrease in the growth of interferon treated cells compared with untreated ones (39), which tells against the hypothesis.

These, then, are the advances in cellular immunology, so vital in an understanding of virus infections, which have been brought about by interferon. This substance would be an ideal antiviral 'antibiotic' (if indeed human cells and viruses can be termed micro-organisms) due to its lack of toxicity and antigenicity. Trials in humans have not been very forthcoming however. The evidence at present is promising, but limited. Interferon prepared in rabbit kidney cells was found to protect rabbits against against intradermal infection with vaccinia virus when given before or at the same time as the virus (36). In a human trial, (37), 38 volunteers were vaccinated at two sites, one which had been injected twenty fours previously with interferon, and the other with control fluid. The trial was carried out and assessed blind. A highly significant degree of protection by interferon was found. (figs. 1,2 over).
Jones et al. have tried monkey interferon in the treatment of vaccinial keratitis, presenting granular epithelial opacification and ulceration (32). The opacification cleared quickly in 24 hours. The ulcer healing occurred at the same rate as in two other cases treated by débridement. However, in both instances, stromal oedema and diffuse infiltration occurred. They concluded that topical application of monkey interferon has a striking antiviral effect, but did not prevent stromal sequela.

So far, no satisfactory evidence is available to show that interferon is effective in established infections. If its mode of action is in fact as thought, there seems no reason why it should not be so. It has been shown to be effective in vitro if given within the first half of the virus growth cycle; and preparations used so far have been both weak and impure. Further trials must be awaited. However, large scale production would be a difficult and costly business, requiring the use of many monkey preparations (which are at present necessary because of the partial cell specificity of interferon). It cannot be given by mouth, as, being a protein, it would be digested.

Steroids have no place in virus infections, except for the symptomatic treatment of rare life-threatening cases of infectious mononucleosis. In most diseases, especially herpes simplex keratitis, their use may lead to an exacerbation of the attack.
Conclusion.

At present, medicine cannot fight the virus in straight forward combat, although she is trying to forge weapons. However, by rendering his abode untenable, she can undermine his assault.

Perhaps an analogy can be drawn between the search for chemical agents to control virus infections and the research into cure for cancer, for both are concerned with corrections of aberrations in the replicating mechanism of the cell. Consequently, viruses are constantly under suspicion as a cause for cancer. But the replicating mechanism of the cell is the core of life itself: is it presumptuous to assume that man can unravel its mysteries completely?

The pioneers in chemotherapy against bacterial infections almost certainly had their doubts. The problem facing virologists to-day is more profound, but their resources and knowledge are correspondingly greater.
References.


Textbooks.
Textbook of virology: Rhodes and Van Rooyen.