An adequate and well-balanced diet forms an essential part in the treatment of all anæmias—i.e., a diet containing fresh red meat, green vegetables, fresh fruit, milk, and butter.

Transfusion

Hæmopoietic substances are often given to other anæmias than those dependent upon a deficiency of such factors. There is no evidence that they are of any value, and they are often only an unjustified expense. Such anæmias may be benefited by blood transfusions. Transfused blood may apparently act in two ways. It may supply sufficient blood to enable the patient to carry on for a week or fortnight but then has to be repeated as in aplastic anæmia. Transfusion also appears in some instances to stimulate hæmopoiesis. At present it is impossible to predict when this will occur. Provided adequate steps are taken to transfuse compatible blood there are, I believe, no contra-indications to transfusion. I have seen many patients benefited, and only one instance in which transfusion could possibly be said to have shortened life. It is always worthy of trial in anæmias which fail to respond to other treatment. Direct matching of the blood is essential, especially in patients who have been frequently transfused.

Splenectomy

The value of splenectomy in anæmia is as controversial as that of transfusion. It is of undoubted value in acholuric jaundice, and gives at least temporary relief in idiopathic purpura. Its value in early cases of splenic anæmia is less certain. In a certain proportion of patients it appears to cause amelioration of symptoms. Splenectomy should not be attempted until iron therapy has failed. Idiopathic microcytic anæmia may simulate splenic anæmia. The one responds to iron the other does not. This affords an excellent example of the value of carefully controlled treatment in the diagnosis of anæmias.

A VIRUS OBTAINED FROM INFLUENZA PATIENTS

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THE epidemic of influenza at the beginning of 1933 afforded an opportunity of making an experimental study of this disease, the results of which are here embodied in a preliminary communication. Throat-washings were obtained from a number of patients as early as possible after the onset of definite symptoms. On the assumption that the ætiological agent of influenza was probably a filtrable virus the throat-washings were filtered before use through a membrane impermeable to bacteria. The filtrates, proved to be bacteriologically sterile, were used in attempts to infect many different species. All such attempts were entirely unsuccessful until the ferret was used and the first success was only secured towards the close of the epidemic.

The initial successful experiment was made with two ferrets, both of which received a filtrate of human throat-washings, both subcutaneously and by intranasal instillation. Both animals became obviously ill on the third day after infection and exhibited symptoms of the characteristic disease which is described below. It was found that the disease could be transmitted either by contact or by direct transference of nasal washings from a sick to a healthy ferret. At this point therefore the work was transferred to the Institute's farm laboratories at Mill Hill, where it could be carried out under the conditions of rigid isolation of individual experimental animals evolved and used by Dunkin and Laidlaw[1] in their work on dog distemper * (1926).

The Disease in Ferrets

The ferret disease is characterised by a two-day incubation period, a diphasic temperature response, symptoms of nasal catarrh and variable systemic disturbances. In the infected animal the temperature† rises abruptly about 48 hours after infection, often exceeding 105° F. or even 106° F. It subsides on the third or fourth day only to rise again on the fourth or fifth day. In the course of the next day or two the temperature gradually returns to normal, and in most cases remains thereafter within normal limits.

Coincidently with the primary rise of temperature the ferret looks ill, is quiet and lethargic, often refuses food, and may show signs of muscular weakness. The catarrhal symptoms usually begin on the third day. The eyes become watery and there is a variable amount of watery discharge from the nose. This nasal discharge at times becomes sticky and may be mucopurulent, thus causing matting of the fur along the edges and at the corners of the nostrils. The animal sneezes frequently, yawns repeatedly, and in many cases breathes partly through the mouth with wheezy or stertorous sounds which clearly indicate a considerable degree of nasal obstruction. Such obstruction rarely accompanies a copious nasal discharge. The tip of the nose is often very pale. The signs of illness may last for only a few days but sometimes continue for ten days, after which the ferret again becomes perfectly normal. There is considerable variation as regards both the temperature response and the intensity and time of appearance of the local symptoms. In a few ferrets a typical diphasic temperature response has occurred without any nasal symptoms, and in one case well-marked symptoms were noted without any elevation of temperature. These animals when tested later were found to be immune. Very occasionally a ferret, a short time after recovery, has had a relapse in which the temperature curve and the symptoms have been similar to those of the primary illness. The disease has never been fatal in the 64 cases observed throughout the full course of the illness. Fig. 1 illustrates the temperature response of a ferret which had a typical attack of the disease, with a relapse.

In ferrets killed during the first and second febrile periods the mucous membrane of the nasal passages shows acute inflammation. Sections across the turbinate bones show, in the soft parts, acute vascular congestion, dilated lymph channels, numerous leucocytes passing out through the epithelium, and serious

* It is essential, when employing ferrets as experimental animals, that all purchased animals be quarantined for 14 days before being brought into use. If this precaution be omitted it is probable that latent distemper infection will, sooner or later, give rise to serious confusion. There is another acute infectious disorder, the ætiology of which is still obscure, which may also give untold worry to the research worker. Neck abscesses may develop in older animals and examination for these is essential if error is to be avoided.

† The normal temperature of a ferret is somewhat variable but it is unusual in a quiet animal to record a reading of over 103·5° F.

derangement of this structure. There is almost invariably complete disappearance of ciliated cells, and occasionally patchy necrosis of the whole thickness of the epithelium may be observed. No histological feature, such as an inclusion body, has as yet been discovered which can be called characteristic of the disease.

Passage of the Virus

The disease has frequently been transmitted by placing a normal ferret in the same cage as a sick one for 24 hours. The majority of virus passages, however, have been made by the following technique.

The infected animal is killed when showing symptoms, often at the beginning of the second temperature rise. The turbinates are scraped out, ground up with sand, and emulsified in about 20 c.cm. of equal parts of broth and saline. The emulsion is lightly centrifuged, and about 1 c.cm. of the supernatant fluid is dropped into the nostrils of another ferret. In this way 26 serial passages of one strain of virus have been made, and every animal of the series has shown the typical temperature response together with definite symptoms of the disease. A hundredfold dilution of the usual preparation has also been found to be regularly infective.

The method we are forced to employ has the serious disadvantage that it is impossible to make accurate quantitative experiments. The concentration of virus in the emulsion is unknown and it is impossible to determine what proportion of the amount instilled into the nose is retained, but no other route of inoculation has yet proved successful and other tissues tested—e.g., spleen, lymph glands, and blood—have been uniformly non-infective.

Throat-washings from eight human cases diagnosed as influenza have been inoculated into ferrets; five of these produced the ferret disease described above, although four were tested before the importance of utilising temperature records was recognised. From one of these cases throat-washings on the first and second day, and nasal discharge on the third day, were infective for ferrets, but on the sixth day no virus was recoverable from the nasal discharges, and on this day there was considerable improvement in the patient's catarrhal symptoms. A filtrate prepared from an emulsion of lung tissue from a fatal case of influenzal pneumonia likewise produced the ferret disease.

Throat-washings from four human subjects not suffering from influenza were non-infective. Of these, two were taken from men who had recovered from influenza and who at the time of their illness had supplied washings which were the genesis of the transmissible strains of virus with which we have done most work.

The nasal secretions of a man who was suffering from a severe common cold were also non-infective.

Filtrability of the Virus

Most of the human throat-washings were filtered before use through membranes having an average pore size of 0·6 μ. The membranes were Gradocol membranes made and supplied by Mr. W. J. Elford, Ph.D.[2] (1931), to whom we are greatly indebted. The filtrability of the virus after ferret passage was tested repeatedly. Invariably filtrates of an emulsion of the nasal mucosa from a sick ferret through membranes having an average pore size of 0·6 μ were found to produce the typical disease. A tight membrane (a.p.s. 0·25 μ) was used on one occasion only; the resultant filtrate was infective. It is probable therefore that the virus of ferret influenza is no larger than the viruses of vaccinia or herpes febrilis.

The infectivity of the filtrates, coupled with the fact that we failed to grow anything from the filtrate on a variety of media under aerobic or anaerobic conditions, has convinced us that we are dealing with a true virus disease. We have examined a number of bacteria from ferrets and human beings and so far we have failed to discover any micro-organism which will mimic the disease when cultures are instilled into a ferret's nose. *Hæmophilus influenzæ*, *Hæmophilus canis*, and *Hæmophilus influenzæ (suis)* administered along with virus produced at most only minor variations in symptoms.

Active Immunity

Ferrets which have recovered from the disease are invariably found to be immune to subsequent infection with the same strain of virus. This holds true whether the immunity test is done a few days after the disappearance of symptoms or five or six weeks later. One of two ferrets tested after the lapse of three months proved to be solidly immune and the other had a very mild attack of the disease with prompt recovery. At the present time it remains uncertain whether viruses from different human sources cross-immunise completely. No means of securing an active immunity apart from giving the disease itself have yet been found.

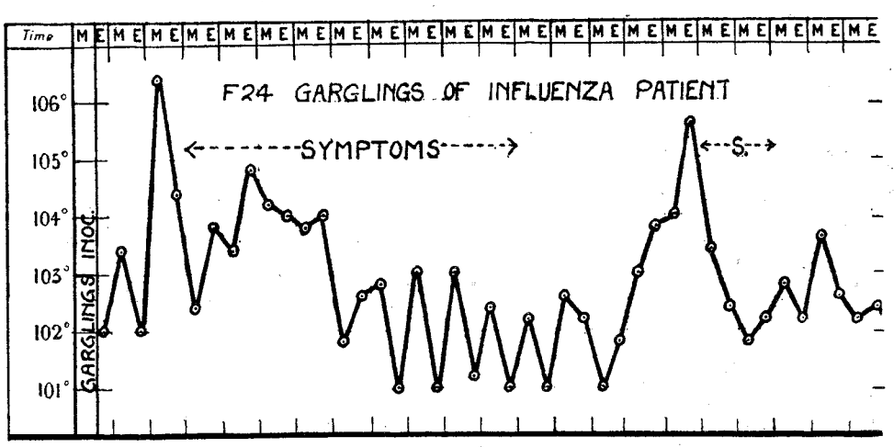

FIG. 1.—Temperature chart of ferret infected with garglings from a patient. The relapse is very unusual.

Virus Neutralisation

The serum of a ferret which has recovered from the disease will neutralise strong emulsions of the virus, provided that the serum and virus are mixed together before being inoculated intranasally into the test animal. Normal ferret serum has no such power of virus neutralisation, even when dilute virus is used in the test. Fig. 2 illustrates a virus neutralisation experiment.

Many human sera are capable of neutralising dilute ferret virus. Sera obtained from ten patients after their recovery from influenza were all found to have neutralising antibodies, but their demonstration was not constant, for two of the sera when retested failed to protect the experimental animals. Such irregularity is probably due to our inability to measure the dosage of virus employed, and it is quite possible,

under the conditions of the only test at present available, that an overwhelming dose of virus is given in one case and not in another. Control ferrets inoculated with virus alone or with virus mixed with normal serum were always included in these neutralisation tests; they invariably developed the disease.

Three human sera from individuals with no history of a recent attack of influenza were also tested: one showed neutralising properties; the other two were inactive. Such a result would be expected in tests on a population shortly after an epidemic of influenza.

Relationship to Swine Influenza Virus

A disease of swine, which arose spontaneously at the time of an influenza epidemic in America, has been described by Shope [3] (1931, 1932). We are indebted to him for samples of the swine influenza virus, and also for cultures of *Hæmophilus influenzæ* (*suis*), an organism which plays an important rôle in the serious and fatal cases of the swine disease. The virus when inoculated intranasally into ferrets gave rise to a disease with diphasic temperature response, and all the symptoms described above—in fact a disease indistinguishable from the ferret disease caused by virus of human origin. The swine influenza virus was also readily transmissible serially through ferrets. In striking contrast to swine influenza the ferret disease was not modified in character when cultures of *Hæmophilus influenzæ* (*suis*) were inoculated together with the virus.

Cross-immunity tests have shown that this swine influenza virus bears a close antigenic relationship to the virus strain of human origin which has been chiefly used in our work. Ferrets after recovery from disease caused by the swine virus proved to be solidly immune to the human strain of virus. Ferrets convalescent from the human virus disease were not completely immune to the pig strain of virus.

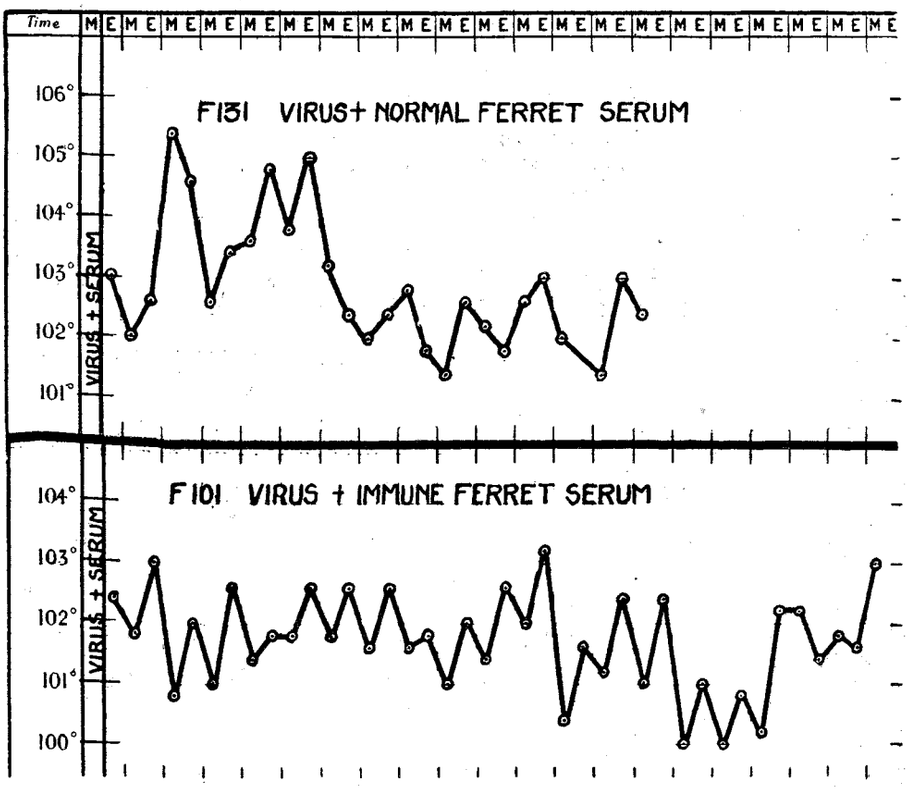

FIG. 2.—Upper temperature chart. Ferret received a mixture of virus with normal ferret serum. Lower temperature chart. Ferret received a mixture of virus with immune ferret serum.

Summary and Discussion

A disease of ferrets, produced by the intranasal instillation of filtrates of throat-washings obtained from influenza patients, is described.

The disease is transmissible serially in ferrets either by contact or by the intranasal instillation of virus-containing material.

The infective agent has, so far, only been recovered from the nasal passages of sick ferrets.

The disease was produced by five of the eight throat-washings obtained from influenza patients in the early stages of the disease.

Throat-washings from healthy persons and influenza convalescents caused no illness in ferrets.

The nasal secretions from a subject with a severe common cold caused no illness in ferrets.

Human sera, particularly those from influenza convalescents, were found to contain antibodies capable of neutralising the virus of the ferret disease.

Swine influenza virus caused a disease in ferrets which was indistinguishable from that produced by virus of human origin, and the pig and human viruses have close antigenic relationships.

We consider that the evidence given above strongly suggests that there is a virus element in epidemic influenza, and we believe that the virus is of great importance in the ætiology of the human disease. This view receives considerable indirect support from the fact that Shope found that the pig virus was the essential factor in swine influenza. The epizoötic disease could only be produced by combining two separate agents: (1) a virus; (2) *Hæmophilus influenzæ* (*suis*). The virus *alone* produced a disease so mild that it was difficult to recognise, and the bacillus *alone* appeared to be harmless. Our results with ferrets, so far as they have gone, are consistent with the view that epidemic influenza in man is caused primarily by a virus infection. It is probable that in certain cases this infection facilitates the invasion of the body by visible bacteria giving rise to various complications. Analogous examples of this type of double infection are seen in swine influenza and dog distemper epizoötics. Decisive evidence on this point, and indeed on the importance of the virus we have described, can, we feel, only be secured by intensive study during an influenza epidemic, since direct experiments on man are fraught with difficulties. We are led to the publication of this preliminary note by the hope that our findings may be of assistance to those, wherever they may be situated, whose fate it may be to study the next epidemic of influenza.

We desire to thank the various practitioners through whose kindness we obtained throat-washings from influenza patients.

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