PROTECTION OF MICE AGAINST THE LETHAL ACTION OF AN ENCEPHALITIS VIRUS

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Although interferon was first found (Isaacs and Lindenmann, 1957) in the course of investigating a laboratory phenomenon, virus interference, subsequent investigations have brought it nearer to virus infections of man. In the first place, interferon has been shown to protect cells *in vitro* against infection with a wide range of viruses, and in one instance—namely, vaccinia infection of the rabbit skin—protection was found *in vivo* also (Isaacs and Westwood, 1959). In the second place, production of interferon has been shown to be a response of many different kinds of cells to infection with a variety of different viruses *in vitro* and *in vivo*, and there is some evidence that both *in vitro* (Ho and Enders, 1959; Henle *et al.*, 1959) and in the animal body (Isaacs and Hitchcock, 1960) production of interferon may have a protective action against virus infection.

If we consider interferon as a protective response of cells to virus infection we can usefully compare it to the production of specific antibody. The antibody response to infection has been exploited in medicine in two different ways. Antibody may be given *passively*—that is, antibody produced in one animal or individual is administered to protect a second animal or individual. Alternatively, an animal or individual may be stimulated *actively* to produce antibody needed for its own protection. By analogy one could imagine that interferon might be used to protect after passive administration, or by actively stimulating an animal to produce enough interferon to protect itself.

In the present experiments mice have been protected against the lethal action of an encephalitis virus (Bunyamwera virus) both by passive administration of interferon and by actively stimulating the mice to produce interferon. The degree of protection achieved has not been great, though it is statistically highly significant. But, in spite of the low degree of protection achieved, the results are reported now, since they emphasize a principle which may be useful in future experimental work.

Preparation of Interferon

In Tissue Culture.—8-oz. (225-ml.) medical flat bottles containing thick sheets of about one-week-old primary mouse embryo fibroblasts were infected with the Kunz strain of influenza A¹ virus diluted 10^{-2} in 12 ml. of Parker's 199 medium. After incubation for three or five days at 37° C. the medium was harvested and dialysed against HCl/KCl buffer at pH 2 for 12 hours at 4° C. to kill the virus. It was then brought back to pH 7.4 by dialysis against two changes of Earle's buffered salt solution at 4° C. This fluid was used as such or after concentration 5 to 10 times by pressure dialysis. Control fluid was made in the same way without addition of virus.

In Mouse Lungs.—Swiss white mice (TO strain) of ages varying from 3 to 8 weeks were inoculated intranasally under light anaesthesia with 0.05 ml. of the PR 8 strain of influenza virus A diluted 10^{-5} in broth saline and containing about 600 egg infective doses of virus. After three days the mice were killed with coal-gas and their lungs removed and ground up in 1 ml. of Earle's saline to each pair of lungs. After centrifuging, the lung extracts were treated at pH 2, as described for tissue-culture interferon. Ten times concentration of lung extracts was accomplished by negative-pressure ultrafiltration. Control extracts were made from normal mouse lungs.

Mouse Protection Experiments

TO mice (about 3 weeks old and weighing 10–12 g.) were used in all experiments, and caged in groups of six.

Protection by Interferon Given Intraperitoneally.—Doses of 0.2 ml. of interferon or control material were inoculated intraperitoneally on two consecutive days. The mice were also challenged on the second day with 0.2 ml. of Bunyamwera virus intraperitoneally. A stock of Bunyamwera virus made in baby mouse brains and stored at -79° C. was used in all experiments. A range of $\log_{10}$ dilutions of virus was used in every experiment in both the control and the interferon-treated mice. In assessing the results, the dilutions of virus which killed 50 to 100% of control mice were picked and the numbers of mice killed by these dilutions in the two groups were compared. A record was kept of the day of death of the mice, and the mean day of death after challenge was calculated for each group.

Protection by Influenza Virus Given Intranasally.—Pools of Kunz strain of influenza virus A were made from embryonated eggs infected by the allantoic route. Infected allantoic fluid was diluted 1/10 in broth-saline and 0.05 ml. inoculated intranasally in mice under ether anaesthesia. This dose contains 10^6 to 10^7 egg infective doses of virus. Control mice were not inoculated. All mice were challenged with Bunyamwera virus as described above and the results assessed in the same way. The influenza virus was given at various days before and after the challenge virus. The Kunz virus did not kill mice when given intranasally at this dosage, but usually the mice became sick two days after inoculation, and, if killed, they could be seen to have influenza lung lesions. They recovered from this infection by the sixth day.

Results

Protection of Mice by Intraperitoneal Inoculation of Interferon

Table I shows the combined results of 11 experiments carried out in an effort to protect mice against encephalitis by the Bunyamwera virus. Mouse-tissue-culture interferon was used in three experiments and

TABLE I.—Protection of Mice by Intraperitoneal Inoculation of Interferon Prepared in Tissue Culture and in Mouse Lungs

Interferon Prepared in	No. of Mice Dead after Intraperitoneal Challenge with Bunyamwera Virus		
	Control	Interferon-treated	P
Tissue culture (3 experiments)	21/36	8/37	<0.01
Infected mouse lungs (8 experiments)	69/90	27/72	<0.01
Total (11 experiments)	90/126	35/109	<0.01

mouse-lung interferon in eight experiments. The combined results of all 11 experiments are shown, and this degree of protection is highly significant at the 1% level.

The average difference between the LD₅₀ (50% lethal dose) of interferon-treated and control mice was log₁₀ 1.0 over five experiments—that is, the interferon treatment reduced the effective lethal dose of virus by a factor of 10 times.

An attempt was also made to see whether interferon would protect mice against challenge by Bunyamwera virus inoculated intracerebrally. This is a much more severe test of resistance, and no protective effect was found in these experiments.

Protection of Mice by Infection with Avirulent Influenza Virus

It has been previously reported that interferon is demonstrable in the lungs of mice after infection with either a small dose of virulent influenza virus or a large dose of avirulent virus. In mice infected in this way the concentration of interferon in the lungs remained high in the first few days of infection but was lower on the seventh day (Isaacs and Hitchcock, 1960). The possibility was considered that interferon, produced in the lungs, might pass into the blood-stream and spread to other tissues and organs. Accordingly, experiments were designed (as described above) to see whether a large dose of influenza virus, which produced interferon in the lungs, would protect mice against intraperitoneal challenge with Bunyamwera virus.

TABLE II.—*Protection of Mice by Intranasal Inoculation of Avirulent Influenza Virus*

Influenza Virus Inoculated Intranasally	Deaths from Intraperitoneal Challenge with Bunyamwera Virus	
	No. of Mice Dead	Mean Day of Death
One day before challenge ..	1/12	9
Day of challenge ..	6/12	8.5
One day after challenge ..	3/12	7.7
Two days ..	7/12	10
Nil ..	10/12	7.6

Table II shows the results of one experiment. These suggest that the influenza virus infection protected the mice when it was given one day before, along with, or one day after the challenge virus.

The mean day of death for the mice which died in the various groups is also shown in Table II. It can be seen that influenza virus infection reduced the number of deaths and delayed the day of death. The pooled results of three experiments, shown in Table III, indicate that influenza virus given one day before challenge induced statistically significant protection in these experiments. Influenza virus given after challenge did not reduce significantly the numbers of deaths in further experiments, but a slight protective effect was indicated by delay in the day of death. Similar experiments were then carried out except that the mice were challenged with Bunyamwera virus by the intracerebral route, but no protective effect could be shown. One experiment was also carried out to see whether a combination of passive inoculation of interferon and active infection with influenza virus would produce greater protection than either separately, but no additive effect was found.

Mice known to be producing interferon in their lungs as a response to influenza virus infection were killed at intervals and an attempt was made to detect influenza virus and interferon in the blood-stream and interferon

in the brains. At no time was any trace of viraemia found, a result which is in agreement with present knowledge of the pathogenesis of influenza. In one experiment a small amount of interferon was demon-

TABLE III.—*Protection of Mice by Intranasal Inoculation of Avirulent Influenza Virus (Pooled Results of Three Experiments)*

Influenza Virus Inoculated Intranasally	Deaths from Intraperitoneal Challenge with Bunyamwera Virus	
	No. of Mice Dead	Mean Day of Death
Day before challenge ..	10/30	8.5
No influenza virus given ..	31/36	7.0
	p<0.01	

strated in both serum and brains at about three days after infection, but in other experiments interferon could not be demonstrated. It appears that if interferon is liberated into the blood-stream it is present there only in small amounts.

14 to 21 days after virus challenge, sera were collected from mice protected by interferon and tested by the technique of Porterfield (1960) to see if they contained antibodies to Bunyamwera virus. In a proportion of animals which recovered, viral antibody was detected in their sera. Further investigations are required on the course of development of antibody in these animals.

Discussion

These experiments have had the very limited aim of trying to see whether it is possible to protect animals against a systemic virus infection by passive administration of interferon and by active stimulation of interferon production. Slight but definite protection has been achieved by both methods, but so far only very limited experiments have been carried out to see whether different dosages and different schedules of administration of interferon would increase the degree of protection.

In addition, little is known of the method by which interferon confers increased resistance in mice. Its antiviral action is probably short-lived, and yet virtually complete protection is achieved in individual animals. This suggests that passively administered interferon inhibits virus growth without inhibiting the natural defences of the body. The natural defences would include production by the infected animal of fresh interferon and antibody. There is already evidence that interferon, in doses which inhibit virus growth, does not inhibit the production of fresh interferon *in vitro* (Isaacs and Burke, 1958). It is not yet known whether interferon affects antibody production, but the finding of viral antibody in mice which had been treated with interferon, infected with encephalitis virus, and which then recovered, is encouraging. It therefore appears that in this situation the interferon acted by limiting the number of cells which would support virus growth, and thus it slowed down the rate of virus multiplication sufficiently to allow time for mobilizing the natural defences of the body.

It was surprising to find that influenzal infection of mice could protect them against an encephalitic infection. Viral interference has, of course, been a well-recognized phenomenon from the time that Findlay and MacCallum (1937) showed that monkeys infected with Rift Valley fever virus were temporarily resistant to the virulent yellow-fever virus, these two viruses being antigenically unrelated. Many similar examples have

been described since then, but it had usually been assumed that the interference was due to occupation of cells by the first virus, leading to exclusion of the second virus. The interest of the present experiments is that the influenza virus remains in the lungs and cannot be demonstrated in the blood-stream, so that the resistance of the mice is probably due to interferon produced in the lungs and diffusing to the other organs via the blood-stream. An analogous explanation has been put forward to account for the observation of Burnet and Fraser (1952) that chick embryos infected with influenza virus in the allantoic cavity became resistant to the lethal action of the neurotropic influenza virus strain NWS. Grossberg, Hook, and Wagner (1960) found that a similar resistance could be induced by inoculating interferon into the allantoic cavity, and they suggested that the "interference at a distance" described by Burnet and Fraser was due to the production of interferon in the allantoic cavity as a result of infection with the influenza virus, followed by diffusion of the interferon into the chick embryo.

The present experiments do not allow us to tell which would be the best route towards the goal of protecting man against virus infections, but they point to a number of interesting avenues of exploration.

Summary

Mice were protected to a slight but statistically highly significant extent against the lethal action of an encephalitis virus. Two different methods were used. The first was by intraperitoneal inoculation of interferon, and the second by active infection with influenza virus. The influenza virus grows in the mouse lung and protects the mice apparently by inducing production in the animals' lungs of interferon, which then diffuses into the blood-stream. It is suggested that interferon may protect the mice by inhibiting the growth of the encephalitis virus without impairing the animals' natural bodily defences.

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REFERENCES

- Burnet, F. M., and Fraser, K. B. (1952). *Aust. J. exp. Biol. med. Sci.*, **30**, 447.
 Findlay, G. M., and MacCallum, F. O. (1937). *J. Path. Bact.*, **44**, 405.
 Grossberg, S., Hook, E. W., and Wagner, R. R. (1960). *Fed. Proc.*, **19**, 408.
 Henle, W., Henle, G., Deinhardt, F., and Bergs, V. V. (1959). *J. exp. Med.*, **110**, 525.
 Ho, M., and Enders, J. F. (1959). *Proc. nat. Acad. Sci. (Wash.)*, **45**, 385.
 Isaacs, A., and Burke, D. C. (1958). *Nature (Lond.)*, **182**, 1073.
 — and Hitchcock, G. (1960). *Lancet*, **2**, 69.
 — and Lindenmann, J. (1957). *Proc. roy. Soc. B.*, **147**, 258.
 — and Westwood, M. A. (1959). *Lancet*, **2**, 324.
 Porterfield, J. S. (1960). *Bull. Wld Hlth Org.*, **22**, 373.

Four times as many overseas students as there were 10 years ago are now in Britain. The estimated total for 1959-60 is 47,500. More than half were living and studying in the London area. These and other facts are contained in a new and expanded edition of a handbook, *Overseas Students in Britain*, published by the Standing Committee of the London Conference on Overseas Students, which represents about 70 organizations concerned with overseas students and has a secretariat provided by the British Council. The booklet may be obtained from the London Overseas Department of the British Council, 3 Hanover Street W.1. (Price 1s. 6d., post free.)

STUDIES WITH ¹³¹I TRIOLEIN IN SOUTH AFRICAN BANTU AND WHITE SUBJECTS, AND IN PATIENTS WITH MYOCARDIAL INFARCTION*

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Recent studies on the levels of blood radioactivity after the oral administration of ¹³¹I triolein have demonstrated significant differences between normal subjects and patients with ischaemic heart disease (Likoff *et al.*, 1958; Sellar *et al.*, 1959). Likoff *et al.* estimated the radioactivity in whole blood and in the trichloroacetic acid precipitable fraction after the oral administration of ¹³¹I triolein to normal males and to males who had suffered a previous myocardial infarction. Significant differences in radioactivity at the peak time and at the 24-hour interval were demonstrated in the two groups. In a similar study, Sellar *et al.* found the blood radioactivity of normal subjects after the oral administration of ¹³¹I triolein to be much lower than in patients with ischaemic heart disease.

Plasma levels of radioactivity after the oral administration of ¹³¹I triolein probably represent a complex resultant of the rates of fat absorption from the bowel and of clearance from the blood. The results in ischaemic heart disease and control subjects would therefore appear to reflect metabolic differences in regard to one or the other mechanism, or to both.

There are well-recognized differences in the dietary and disease patterns in the South African White and Bantu populations. While the consumption of fat and the incidence of ischaemic heart disease in Whites are probably comparable to those of the people of Western Europe and the United States, the average Bantu diet contains little fat (Walker and Arvidsson, 1954), and myocardial infarction is rare. Although this rare incidence of ischaemic heart disease among the South African Bantu may be a reflection of constitutional differences, habitual dietary factors, especially the type and amount of fat consumed, may also be of significance.

Material and Methods

In the present study the absorption and plasma clearance of a test dose of ¹³¹I triolein was followed in Bantu and White subjects having no evidence of ischaemic heart disease, and in White patients convalescing from an attack of myocardial infarction.

Bantu Subjects.—29 male hospital patients aged 40 to 60, convalescing from diseases not generally affecting absorption from the gastro-intestinal tract, and with no evidence of ischaemic heart disease.

White Control Subjects.—30 male hospital patients aged 40 to 60. There was no evidence of any disease affecting gastro-intestinal absorption, or ischaemic heart disease.

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