yield of 4-fluoro-3-nitrophenyl azide (m.p. 52° C) as light-sensitive straw coloured needles (from light petroleum, b.p. 40°–60° C). Treatment of this compound with aqueous methylamine gave 4-azido-(N-methyl)-2-nitro-aniline (m.p. 122°–123° C) which had an ultraviolet absorption maximum at 460 nm (ε 4,800).

The antigen was prepared in the dark because the NAP group is sensitive to light. Bovine γ-globulin (BGG 1 g) was dissolved in borate buffer, *p*H 9·8 (100 ml.), and the solution was treated with 4-fluoro-3-nitro-phenyl azide (1·0 g) in acetone (10 ml.). The reaction mixture was stirred at 40° C overnight and then filtered. The filtrate was dialysed against water for three days, filtered and then freeze-dried to give NAP–BGG (about 1 g) as a pink, light-sensitive product.

The number of NAP groups per mole BGG was determined by analysis of residual lysine residues, by measurement of absorption at 460 nm, and by measurement of N_2 evolution during irradiation. All three determinations indicated approximately 60 NAP-lysine residues/mole BGG.

Twelve rabbits were given a primary subcutaneous injection of 5 mg NAP–BGG in Freund's adjuvant. A booster injection was given intravenously either with 1 mg NAP–BGG in adjuvant or with 1 mg of alum-precipitated antigen; the second method gave a marginally better response. The animals were bled over a period of 4 months, with intermittent booster injections when assays of the sera indicated that they were needed. The antibody response was 400–800 μg/ml. of antibody precipitating with NAP-human serum albumin, and the antibody was therefore assumed to be specific for the haptenic group.

The antibodies were precipitated (according to the method of Eisen[5]) with either NAP–BGG or NAP–HSA and the resulting precipitate dissolved overnight at 37° C in a saturated solution of 4-azido-2-nitrophenol (Fig. 1, if $R = OH$) in sodium carbonate–sodium bicarbonate buffer, *p*H 9. The resulting solution was passed down a DEAE-cellulose column to remove antigen and haptene. (4-Azido-2-nitrophenol was prepared by treatment of 4-fluoro-3-nitrophenyl azide with sodium hydroxide.)

The isolated anti-NAP antibodies gave a single slow-moving electrophoretic band on cellulose acetate, a single symmetrical peak $s_{20w} = 6{\cdot}3$ on ultracentrifugation, and single line in double diffusion against goat anti-whole rabbit serum. These antibodies thus seem to contain only rabbit IgG molecules.

The radioactive haptene, ε-NAP-[4,5-$^3H_2$]L-lysine (NAP-[^{3}H]lysine) (Fig. 1; R = ε-amino-L-lysine) was prepared by treating a solution of 4,5-[$^3H_2$]L-lysine monohydrochloride in sodium carbonate–sodium bicarbonate buffer (*p*H 10·8) with an equimolar amount of 4-fluoro-3-nitrophenyl azide in acetone. The reaction mixture was stirred overnight at 40° C. After extraction with ether, NAP-[^{3}H]lysine was purified by thin-layer chromatography on silica gel plates developed with diethyl ketone-water.

The affinity constant of the anti–NAP antibody with NAP-[^{3}H] lysine as measured by equilibrium dialysis[6] was $6{\cdot}7 \times 10^6$ l./mole at 4° C.

Anti-NAP antibody (3 mg/ml.) was treated with a five-fold molar excess of NAP-[^{3}H]lysine at 4° C in 0·03 M phosphate buffer (*p*H 7·4) and left for 2 h in the dark; the mixture was then passed down a 'Sephadex *G*-25' column which had previously been equilibrated with 10^{-7} M NAP-[^{3}H]lysine in the same buffer, and the protein fraction collected. The protein had absorbed approximately 2 moles of NAP-[^{3}H]lysine per mole of antibody. The antibody–haptene conjugate thus prepared was irradiated at 4° C for 18 h by two Mazda 125 W *MB/V* pearl glass lamps immersed in a solution of sodium nitrite (to absorb any radiation of shorter wavelength than 400 nm).

IgG was added as carrier to an aliquot of the resulting reaction mixture and the protein was precipitated with trichloroacetic acid; the precipitate was washed and then dissolved in 0·1 M sodium hydroxide and the radioactivity in this solution determined. This method indicated that 1·1 moles of NAP-[^{3}H]lysine was bound per mole of protein. In a second experiment 1·2 moles haptene was bound per mole protein.

When the affinity constant of the reacted antibody for NAP-[^{3}H]lysine was remeasured, it was found to be too low to obtain a satisfactory figure. The association constant was certainly less than 10^4. The rather low value of bound haptene per molecule in unreacted antibody was probably a result of partial blocking of the sites with nonradioactive haptene during isolation of the antibody, and of the presence in the radioactive haptene of a small proportion of molecules which had been accidentally activated by light before the reaction. When radioactive NAP-lysine was diluted with a twenty-fold excess of nonradioactive haptene no significant radioactive labelling could be detected, and if inert rabbit IgG was used in place of antibody no labelling occurred. Hence it seems that the photogenerated nitrene is localized specifically within the antibody combining site.

After a total reduction with dithiothreitol in 6 M guanidine[7] the reacted antibody was dissociated into its peptide chains by chromatography on a 'Sephadex *G*-200' column in 5 M guanidine. The ratio of label was found to be 3·5/1·0 in the heavy/light chains respectively. In a preliminary experiment, treatment of the heavy chain with cyanogen bromide[8] showed no significant labelling in the peptides from the C-terminal half of the chain. It seems therefore that this is a satisfactory method of positioning a covalently bound reagent specifically in the part of the peptide chains which form an antibody combining site. Because most of the amino-acid sequence of the N-terminal half of the heavy chain of rabbit IgG has been determined[9] it should be possible to define the section or sections concerned.

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Production of the Influenza Syndrome in Man with Equine Influenza Virus

An antigenic relationship has been established between A/equi-2 influenza virus isolated in 1963 and the 1968 Hong Kong variant of human type A_2 influenza virus[1]. This relationship suggests that the Hong Kong variant was derived from the equine virus. To evaluate this relationship further and to test the possibility of an exchange of viruses between species we have carried out virus challenge studies with normal volunteers. Here we describe the occurrence of clinical syndromes similar to those described for naturally occurring influenza after inoculation of antibody-free volunteers with a strain of influenza A/equi-2 virus.

We used influenza A/equi-2/Miami/1/68, a strain originally recovered in fertile hen's eggs from a naturally infected horse. The inoculum we used was also passaged in human embryonic kidney tissue cultures (HK) and in volunteers[2]. The final inoculum had the following passage history: egg_5 HK_2 man_1 HK_4 man_1 HK_3 (ref. 2). After preparation, the inoculum was stored at −70° C and an aliquot was shown to be free of extraneous viruses by methods described earlier[3].

A 1 : 20 dilution of the inoculum was given to six volunteers in March 1965 by nasal instillation and spray into the nasopharynx with a Devilbiss No. 127 atomizer[2]. A simultaneous titration of that inoculation in rhesus monkey kidney tissue cultures (RhMK) revealed that the dose administered was $10^{5\cdot3}$ 50 per cent tissue culture infectious doses ($TCID_{50}$). Virus was recovered from four of these individuals; serum antibody increased in five but there was no illness.

The work described here was carried out in November 1968. Vials of inoculum, stored at −70° C since 1965, were thawed, and 2 ml. of undiluted virus was immediately inoculated into each of fifteen antibody-free prisoner volunteers in the way we have described. Thus there was no possibility of contamination of the inoculum with the Hong Kong variant of type A_2 influenza virus. Simultaneous titration of this inoculum in RhMK revealed an administered dose of $10^{6\cdot75}$ $TCID_{50}$. Before inoculations were carried out the health of the volunteers was established and their written consent was obtained.

Throat swab and gargle specimens were obtained on each of days 0 to 6, 8, 10 and 11 and 0·4 ml. of each specimen was tested for virus in RhMK. Neutralizing antibody titres were determined on heat inactivated (56° C for 30 min) sera using the haemadsorption–inhibition method against 32 $TCID_{50}$ of virus[4]. Physicians looked for signs of illness twice daily.

The inoculated virus was recovered from all volunteers (one to nine positive specimens per man, mean of five; 1 to 6 positive days, mean of 3·3), and fourteen of the fifteen developed a four-fold or greater neutralizing antibody response to A/equi-2 virus in sera collected 4 weeks after inoculation. Thus all fifteen men were infected with A/equi-2 influenza virus. In addition, nine of them developed a four-fold or greater response to the Hong Kong variant of Influenza type A_2. The relationship between the magnitude of the antibody response to A/equi-2 and the Hong Kong variant of influenza is shown in Fig. 1, which suggests that the magnitudes tended to be similar (Spearman's rank correlation test, $r = 0{\cdot}4$, $P < 0{\cdot}05$).

Thirteen of the fifteen volunteers became ill on the second or third day after inoculation. A case report of the most common illness response is shown in Fig. 2. On the second day this volunteer developed a sudden headache,

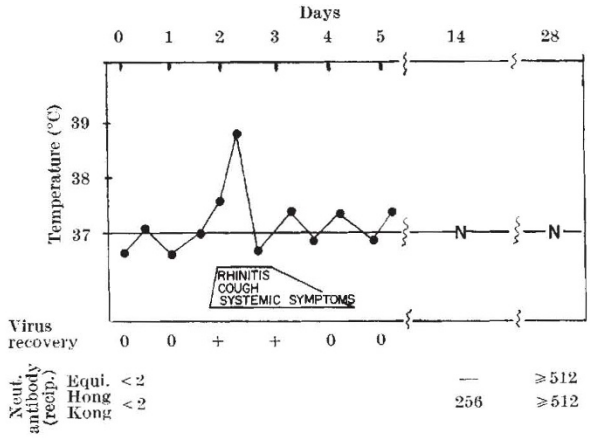

Fig. 1. Case history of a 26 yr old volunteer after inoculation with A/equi-2 influenza virus.

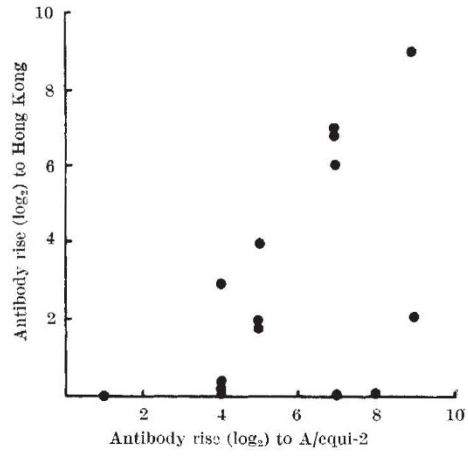

Fig. 2. Neutralizing antibody responses to injection with A/equi-2 influenza virus.

malaise, myalgias and weakness which was soon followed by fever rising to 38·8° C, measured orally. Rhinitis manifested by nasal obstruction and discharge developed within a few hours of the onset of illness and 1 day after the onset he also developed a non-productive hacking cough. Fever lasted for 18 h and during the subsequent 2 days his symptoms resolved so that by the fifth day he was considered clinically well. Isolations and serum neutralizing antibody response to A/equi-2 and the Hong Kong variant of type A_2 influenza virus are shown in Fig. 2.

The pattern of illness responses of all fifteen volunteers is shown in Table 1. The most common response in eight of the volunteers was a febrile systemic and upper respiratory illness (Fig. 1). Two volunteers also exhibited significant symptoms of lower respiratory illness—paroxysmal episodes of coughing, substernal discomfort and tenderness of the trachea. One of these volunteers also experienced a relapse of these symptoms 10 days after inoculation. Three volunteers suffered febrile upper respiratory illness only, and two did not become ill in spite of proven infection. When symptoms and signs were tabulated and frequencies compared with those reported for naturally occurring influenza A illnesses[5], it was noted that fever was of shorter duration (mean 1·3 days), nasal obstruction and discharge were slightly more frequent, and there was a slightly less frequent occurrence of cough and sore throat among the volunteers with equine virus infection. These differences may be a result of the method of inoculation used for equine virus, for it is known that most of the virus deposited in the nasopharynx. But the sudden onset of febrile systemic illness with rhinitis and cough, the most common syndrome in these volunteers, is consistent with descriptions of naturally occurring human influenza[5].

Possible explanations for the illness of these volunteers and not those inoculated earlier are a difference in dose, loss of virus inhibitor with prolonged storage, different environmental conditions at the time of inoculation, and different time of year. An additional question is the part prior passage of the virus strain in man and tissue

Table 1. ILLNESS RESPONSE OF ANTIBODY-FREE VOLUNTEERS TO INOCULATION WITH A/EQUI-2 INFLUENZA VIRUS

	No. of men
Febrile URI†, LRI‡, and Syst§	2*
Febrile URI and Syst	8
Afebrile URI	3
No illness	2
Total	15

All volunteers were infected with the virus.

* One volunteer had a second febrile episode on the tenth day after inoculation.

† Upper respiratory illness.

‡ Lower respiratory illness.

§ Systemic illness.

cultures may have played in producing the illness responses. In previous inoculations, however, passage in man and human tissue culture did not increase the frequency of infections or enhance the virulence for man beyond that seen in the initial inoculum which had been passaged in egg and HK only. This suggests that passage in man did not contribute to virulence. In spite of these considerations the essential point is that an influenza virus of equine origin produced typical human influenzal illness which lends credence to the possibility that in natural conditions equines and equine influenza viruses might contribute to the emergence of new variants of human influenza.

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Seasonal Dormancy in Tea (*Camellia sinensis* L.)

Tea is cultivated between latitudes 45° north and 35° south, and at or near the equator it produces almost the same yield every month. But farther from the equator the winter harvest gradually declines and at latitudes beyond about 16° there is almost complete winter dormancy which lasts longer with increasing latitudes.

The cause of winter dormancy cannot be ascribed solely to low temperature because tea flushes throughout the year at high elevations near the equator, although temperatures there are even lower than winter temperatures in the plains of north-east India[1] (25°–27° N), where bushes are dormant for about 3 months. Soil water stress and nutrition can also be ruled out as major causes of dormancy because even with an ample supply of water and nutrients throughout the dry winter months tea bushes fail to flush at Tocklai (26° 47′ N).

Monthly crop distributions and average day lengths for several tea growing regions from the equator to 30° north or south indicate that tea bushes pass through a period of complete dormancy when the winter day is shorter than a critical length of about 11 h 15 min for at least 6 weeks. The longer the period of short days, the longer the dormancy.

To test this suggestion experimentally, pruned and unpruned mature tea bushes were exposed to 13 h days from November 15 to March 21 by providing weak supplementary artificial illumination during the morning and evening. The intensity of illumination from incandescent lamps in the visible part of the spectrum was less than 1 per cent of the visible solar radiation on a bright summer day and was considered too weak to affect net photosynthesis. Half the experimental area was irrigated with 50 mm of water every month from November to March and the other half was not irrigated. Four similar plots were left as dark controls. Irrigation was discontinued after March, but rainfall in April and May was sufficient to keep the soil fully moist.

Such shoots as were available were harvested from the unpruned plots throughout the winter season and subsequently during April and May. The results showed that irrigation alone increased the winter crop by about 40 per cent and illumination increased it by another 45 per cent. Illumination did not increase the winter crop on unirrigated plots, although it must have induced early bud break as the crop harvested from this plot during April and May clearly shows. With the advent of long days dormancy was also broken in the unilluminated plots, but because of the early start the illuminated plots produced more crop in April–May.

In mid January the pruned and illuminated bushes had twice as many new leaves as the corresponding unilluminated bushes.

In north-east India the tea plant flowers from November to January. Illumination during this period reduced the number of flowers from 376 to 65/m² of unpruned bush surface.

These results demonstrate that increasing the day to 13 h during winter, using weak supplementary artificial illumination, enhanced shoot growth, hastened bud break and inhibited flowering.

If winter dormancy of tea induced by short days is the result of a decrease in the relative concentration of gibberellic acid (GA) in the plant, it should be possible to break dormancy and induce growth in short day conditions by increasing the amount of GA in the dormant plants. This possibility was tested by injecting 10 p.p.m. and 40 p.p.m. respectively of GA into young, single stemmed tea plants on December 30 (1027 h), when all tea plants at Tocklai were completely dormant. As well as GA, kinetin was injected into dormant tea plants to investigate whether it directly or in combination with GA affected bud break and subsequent growth. (Kinetin has been reported to inhibit IAA activity[2].) The compounds were injected into three plants each of two clones by the leaf-stalk injection method[3].

By the middle of February, the plants supplied with GA completed a flush of growth consisting of two to four leaves when other plants were still dormant. These treated plants started the second flush growth in early March, coinciding with bud break and the first flush growth of the untreated plants and those treated with kinetin. The plants treated with GA completed second flush growth by early May.

Clearly, plants treated with GA grew in the short day conditions of winter while untreated plants remained completely dormant. Thus gibberellic acid replaced long days as it did in the case of *Camellia japonica*[4] and *Pinus elliottii*[5]. Kinetin in the concentrations tried had a significantly antagonistic effect on GA activity.

Winter dormancy of tea therefore seems to be a matter of short day length (or long night length), acting through internal plant growth regulators, and it cannot be broken by the conventional methods of commercial management, but the results reported here open up the possibility of inducing growth in short day conditions either by artificial illumination or by treatment with gibberellic acid.

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