and January–March, 1959, respectively (Spicer, 1961). Using suckling mice, the corresponding rates for group B viruses were 20.4, 16.2, and 0 per thousand for the same quarters, results similar to those using tissue cultures. When, however, the Coxsackie A isolations using suckling mice are included, rates of 107, 76.4 and 20.6 per thousand are obtained. The increasing recognition of this group of viruses, together with their ubiquity, constitutes a strong argument for the routine diagnostic use of suckling mice.

The group A viruses isolated belonged to types 2, 4, 5, 6, 8, 10, and 12. Of these, types 5, 6, 8, and 12 have not previously been reported in this country. Types 2, 4, 5, 6, 8, and 10 have been commonly associated with herpangina and their aetiological relationship to this condition has been clearly established (Huebner *et al.*, 1951; Cole *et al.*, 1951; Beeman *et al.*, 1952a, 1952b). Herpangina, as originally described by Zahorsky (1920), appears to be surprisingly uncommon in this country, considering the frequency with which the causative agents have been found. Johnsson (1955) has also commented on the rarity of the herpangina syndrome in his series of patients from whom group A viruses were isolated in Sweden. The finding, however, of these viruses in an apparently normal section of the population serves to emphasize the necessity for caution in attributing to them aetiological significance, even when supported by serological evidence of infection.

Although more than 95% of the Coxsackie viruses isolated in this investigation were from specimens collected between June and December, 1958, occasional isolations were made during the first five months of 1959. It seems probable that the viruses persist in the community throughout the year and spread in the summer and autumn when conditions become favourable. This view is also supported by the apparently simultaneous appearance of the viruses in a number of areas, although in a metropolis such as London, where dissemination may be rapid and widespread, such evidence can carry little weight.

The incidence of Coxsackie infection in the different age-groups suggests that these viruses are very common and are encountered early in life. Beeman *et al.* (1952c) showed by serological investigations that adults had neutralizing antibodies to most of the types against which their sera were tested, whereas children had antibodies to fewer types. The protection afforded by maternal antibody is probably responsible for the low incidence of infection during the first year of life.

An analysis of the carrier rates according to sex was made because of the unexpected finding that the carrier rate of type I poliovirus was significantly higher in males than in females (Spicer, 1961). The carrier rates of Coxsackie viruses of groups A and B, however, showed no significant sex difference.

Summary

A survey was made over a one-year period beginning in June, 1958, to determine the incidence of Coxsackie virus infection in normal children aged 0 to 5 years in the greater London area.

Faecal extracts from 2,084 children were examined by the inoculation of suckling mice and 138 Coxsackie viruses isolated of which 112 belonged to group A and 26 to group B. The viruses isolated included types 2, 4, 5, 6, 8, 10, and 12 of group A and types 3 and 4 of group B. No record has been found in the literature of the

previous isolation in this country of types 5, 6, 8, and 12 of group A.

The monthly isolation rates showed a seasonal prevalence with its peak in August, September, and October, but some types of viruses persisted at a low rate throughout the winter.

The carrier rate was lowest in the first year of life and highest in the second and third years. There was no significant sex difference in the carrier rates.

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ABSENCE OF INTERFERON IN LUNGS FROM FATAL CASES OF INFLUENZA

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Interferon is a potent antiviral substance which is produced by virus-infected cells both *in vitro* and *in vivo* and which is thought to be important in recovery from viral infections (Isaacs and Hitchcock, 1960; Baron and Isaacs, 1961). We have recently developed a quantitative assay for human interferon, using primary cultures of human thyroid cells, and this has made it possible to study the production of interferon during viral infections of man. Through the co-operation of four laboratories we obtained human lung tissues from patients with fatal influenzal pneumonia. Influenza virus had been isolated from all these samples, which had then been stored at low temperatures.

To our surprise there was no detectable interferon activity in any of the specimens we have tested. This paper reports the results and discusses their possible significance.

Materials and Methods

Human lung specimens were generously supplied by Dr. J. Mulder, Leyden; Dr. N. R. Grist, Glasgow; Dr. J. Hambling, London; and Miss E. Minuse, Ann

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Arbor. All specimens were made up as 10%–20% lung extracts which were dialysed to pH 2 and back to pH 7 in order to destroy influenza virus.

Thyroid cell cultures were prepared by mincing surgical specimens from cases of "goitre," trypsinizing overnight at 4° C. in 0.25% Difco trypsin, and growing the cells in medium 199 containing 10% calf serum. The agar overlay medium was composed of Earle's balanced salt solution containing Eagle's nutrients (Eagle, 1955), 0.5% lactalbumin hydrolysate, 15% skim milk, 1:30,000 neutral red, and 1.5% Noble's agar. All media contained 100 units/ml. of penicillin and streptomycin. Interferon was prepared from human thyroid cultures inoculated with a 10⁻² dilution of Newcastle disease virus and incubated at 36° C. for two days. At this time the cells appeared normal and the fluids were harvested and treated at pH 2 in order to destroy residual virus. The pH was lowered to 2 (red colour of thymol blue) by adding 1/3 N hydrochloric acid in the presence of 1/100,000 thymol blue. The acidified fluid was poured into a fresh vessel and the pH raised to 7.8 (blue colour of thymol blue) with 1/3 N sodium hydroxide.

Interferon from human cells was assayed by diluting the interferon preparation in medium 199 containing 15% skim milk, applying 1 ml. of each dilution to culture tubes of human thyroid cells, and incubating on a roller drum at 36° C. After 24 hours the interferon was decanted, the tubes were rinsed three times, and 1 ml. of a 10⁻⁴ dilution of vaccinia virus was applied. After incubation on a roller drum for two hours at 36° C. the fluid was decanted and a thin layer (shell overlay) of nutrient agar was applied by adding 2 ml. of overlay medium and rotating the tube in a horizontal position under flowing cold tap-water. The tubes were then incubated at 36° C. in a stationary position while shaded from light, and vaccinia plaques were counted between the third and fifth days. The dilution of interferon giving 50% inhibition of plaque number was taken as its titre.

Results

Assay of Human Lung Specimens for Interferon

Specimens of human lung which had been stored for different periods under conditions known to preserve interferon were provided by four laboratories. In each case the diagnosis of an influenzal cause of the pneumonia had been confirmed by isolation of influenza virus from the specimens we received. In order to test for interferon

a 1/10 dilution of the 10%–20% lung extract was used in the assay. As may be seen in Table I, all 11 samples were observed to be free from interferon activity at the dilution tested, while the standard preparation gave a titre of 1/1,000.

In an additional two specimens a low degree of antiviral activity was found. However, with further investigation it was realized that both specimens contained antibody to vaccinia virus which was used as the challenge virus in the assay for interferon. This conclusion was based on our finding a greater inhibition of virus when lung extract and virus were incubated together (as in an assay for antibody) than when virus was applied to cells which had been previously incubated with lung extract and then washed (as in an assay for interferon). Since the antibody could not be completely washed out of the culture by five washings with Earle's balanced salt solution, even after application of lung extract to cells for only five minutes, it was not possible to assay for interferon. A similar firm binding of horse serum protein to cell cultures has been reported by Habel (personal communication).

To exclude the possibility that the absence of interferon in the lung extracts might be due to inhibition or inactivation of interferon by a substance in the lung specimens, 100 50% virus inhibitory doses of human interferon (in 0.1 ml.) were incubated for one hour at 36° C. with 0.1 ml. of undiluted lung extract and then diluted to 1 ml. before application to thyroid cultures in an assay for interferon. Two extracts were tested in this manner. The antiviral activity of the interferon mixed with lung extract was equal to the activity of the interferon alone, indicating that the extracts did not inhibit interferon.

Multiplication of Interferon-treated Cells

In a previous communication it was noted that interferon showed less antiviral action in cancer cells and in the cells of young embryos than in normal adult cells. The suggestion was put forward that interferon might play a part in regulating normal cell growth and that its antiviral action might represent a later development in the course of evolution (Isaacs and Baron, 1960). On this hypothesis one might expect that large doses of interferon would inhibit the growth of normal adult cells, and this possibility was tested in human thyroid cells.

Human interferon in a dose of 100 50% virus inhibitory doses was added to cultures of thyroid cells

TABLE I.—*Interferon Assay of Human Lung Extracts from Fatal Influenzal Pneumonia*

Preparation	Source	Age of Patient	Diagnosis	Influenza Virus Type Isolated	Duration of Illness (Days)	Titre of Interferon
Extract T-1881-61 ..	Dr. N. R. Grist, University of Glasgow	48	Influenzal pneumonia. Staphylococcal bronchopneumonia	A2	3	< 10
.. AA-389 ..	Miss E. Minuse, University of Michigan	57	Chronic heart failure	A2	3	< 10
.. AA-391 ..	" " "	79	Multiple cerebral infarcts plus pulmonary atelectasis	A2	11	< 10
.. AA-649 ..	" " "	59	Congestive heart failure	A2	4	< 10
.. C-V-411-61 ..	Dr. J. Hambling, Central Public Health Laboratory, Colindale	83	Influenzal pneumonia	A2	3	< 10
.. C-V-412-61 ..	" " "	83	" "	A2	5	< 10
.. C-V-413-61 ..	" " "	56	" "	A2	4	< 10
.. L-II ..	Dr. J. Mulder, Academisch-Ziekenhuis, Leyden	60	" "	A2	2	< 10
.. L-IV ..	" " "	39	" "	A2	3	< 10
.. L-V ..	" " "	15	Influenzal pneumonia. Staphylococcal bronchiolitis	A2	4	< 10
.. L-VI ..	" " "	39	Influenzal pneumonia	A2	1	< 10
Standard preparation of human interferon from thyroid cultures	" " "	—	—	—	—	1,000

24 hours after they had been seeded into test-tubes. Growth of cells was observed daily and compared with that of control cultures. The experiment presented in Table II shows no gross difference between the growth of interferon-treated and control cultures.

TABLE II.—*Multiplication of Human Thyroid Cells in the Presence or Absence of Interferon*

Growth Medium	Estimated Percentage of Surface Covered by Cells* on Day after Interferon				Vaccinia Virus Plaques per Tube
	0	1	2	3	
199 + 10% calf serum	5-15	25-35	75-80	100	90, 95
100 units interferon + 199 + 10% calf serum	5-15	25-35	75-80	100	0, 3

* Average of two cultures.

To confirm that the interferon was acting throughout the experiment the cultures were tested for sensitivity to vaccinia virus after they were fully sheeted. As is also shown in Table II, the cells which were grown in the presence of interferon were highly resistant to viral growth, indicating that the interferon protected the cells from virus growth but did not prevent their multiplication.

Similar results were obtained in two other experiments with human thyroid cells and also with fibroblasts from mouse embryos near birth.

Discussion

The finding that interferon was not present in extracts of human lung from 11 fatal cases of influenzal pneumonia may be highly significant but is difficult to interpret because similar extracts from survivors of influenzal pneumonia were not available to serve as controls. Nevertheless, since such control material is not likely to become available it may be worth while to attempt an interpretation with the use of less direct information. From the present study and from a previous report (Sutton and Tyrrell, 1961) it is known that human cells are able to produce interferon in high titre *in vitro*. It seems reasonable to infer that in man, as in animals, after stimulation by virus *in vivo*, human lung cells should be capable of producing interferon which could be readily detected by the present very sensitive assay.

It is unlikely that the specimens studied were not representative of an infected portion of the lung, and influenza virus was isolated from all the extracts. From similar specimens from fatal cases of influenza Hers *et al.* (1958) and Hers and Mulder (1961) have demonstrated specific viral lesions and viral antigens in the alveolar cells of the lungs. Even if 1% of each lung sample were infected we would have expected that sufficient interferon would be produced to be detected in this test. This estimation is based on the observation that as much or more interferon is produced by mouse lung *in vivo* than by mouse cells *in vitro* (Isaacs and Hitchcock, 1960). It would seem unlikely that any interferon activity in the specimen was destroyed or masked, since the lung specimens were properly stored and since two of the lung extracts were unable to inhibit added interferon. If we tentatively accept that substantial amounts of interferon may be expected in human lung specimens from cases of influenzal pneumonia that recovered, then it is tempting to speculate that the absence of interferon in the present specimens may be

related to the fatal outcome of these infections. One wonders whether an actively functioning interferon mechanism could under certain physiological conditions be greatly inhibited, possibly through the production of excess cortisone, which has recently been shown (Kilbourne *et al.*, 1961) to inhibit the production and action of interferon.

The possibility that interferon may normally be a regulator of cell growth was suggested by the absence of interferon activity during part of the period of rapid embryonic growth and by the decreased activity of interferon in tumour cells which may be presumed to be free of normal growth regulation. The present results do not support the hypothesis that interferon may be a regulator of cell multiplication; however, a more subtle control of cell growth by interferon has not been excluded. In addition we are left with the problem of how interferon can interrupt the synthesis of virus without preventing the formation of the nucleic acids and proteins required in the multiplication of normal adult cells.

Summary

A quantitative assay for human interferon is described. Analysis of lung specimens from 11 fatal cases of influenzal pneumonia revealed a complete absence of interferon. The implications of this finding are discussed.

The possibility that interferon was a normal regulator of cell growth was studied by observing the growth rate of interferon-treated cells and untreated cells. No difference could be seen between the growth rate of interferon-treated cells and control cells in stationary cultures.

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"Whilst the importance of infant consultations at welfare centres continues to decline slowly as general practitioners progressively take up more of this work and mothers with a progressively better educational background profit from numerous relatively new sources of information not generally available before the war, some aspects of the Department's work are gaining considerably in importance. Unmarried mothers applied in increasing numbers for assistance. West Indian women numbered more than half the British women, while there were more alleged West Indian putative fathers than British. Children requiring taking into care by the Local Authority (a Children's Committee responsibility) again increased very much in number, and, by the end of the year, the percentage of coloured children in care had risen to 14.8." (*Report on the Health of Birmingham in 1960*, Dr. Matthew Burn.)