VIROLOGICAL STUDIES IN NATURAL COMMON COLDS IN SHEFFIELD IN 1960

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It has been known for several years that certain nasal washings derived from people with natural common colds were capable, on serial passage through human volunteers, of producing an experimental infection resembling coryza in a variable number of cases (Andrewes, 1949; Roden, 1958). Hitchcock and Tyrrell (1960) and Tyrrell and Parsons (1960) have shown that nasal washings capable of transmitting infection produced interference with known viruses or cytopathic changes in tissue cultures grown under rather unusual environmental conditions—that is, low temperature and low bicarbonate concentrations. Studies with these viral agents showed that they bore a definite aetiological relation to the experimental disease under study. There appeared to be two distinct categories of this "Salisbury" group of viruses, one producing cytopathic change only on human embryonic kidney tissue, the other producing changes on both human embryo and rhesus monkey kidney cultures.

These findings are of undoubted importance in the interpretation of trials on human volunteers in a defined experimental model infection. However, the relevance of these studies to the aetiology and epidemiology of natural common colds in the community at large can be evaluated only if it can be shown that viruses akin to the Salisbury group can be isolated direct from natural infections at the present time in other localities, or that serological evidence can be obtained to show that such viral agents have a definite role in "wild" colds.

The purpose of the present communication is to report the isolation from cases of natural common colds in Sheffield in January–June, 1960, of cytopathic agents closely resembling the prototype Salisbury strains in their biological characteristics.

Materials and Methods

Tissue Cultures

Monkey kidney (M.K.) cultures were obtained from immature rhesus monkeys (4–6 months old). Human embryo kidney (H.E.K.) cultures were derived from specimens obtained either by hysterotomy or by spontaneous abortion between the 12th and the 28th week of gestation. The preparation of primary tissue cultures from each source was essentially similar. The renal cortex was chopped into small fragments and washed repeatedly in phosphate-buffered saline (P.B.S.) containing 1,000 units each of penicillin, streptomycin, and polymyxin B. A dispersed cell suspension was prepared by incubation at 37° C. with stirring in serial aliquots of trypsin (0.25% "Difco" 1/250 trypsin in P.B.S.). The final washed cell mass was suspended in growth medium at a concentration of 1 ml. of packed cells per 250 ml. of medium. (This is roughly equivalent to 10^5 cells per ml., but, since optimum

suspensions are those containing small clumps rather than single cells, quantitation with counting-chambers is of no great value.)

The cell suspensions were distributed in 1-ml. amounts in 6 by 5/8 in. (15 by 0.6 cm.) "pyrex" test-tubes with silicone bungs or in 50-ml. amounts in 20-oz. (570-ml.) medical flat bottles. Large semiconfluent islets of epithelial tissue were present four to five days after distribution and most cultures were fully confluent by the seventh day. When required for virus inoculation the growth medium was exchanged for 1-ml. amounts of maintenance medium.

Secondary cultures of monkey kidney tissue were made either from bottles of primary tissue prepared as above, or from similar preparations (Mair and Tobin, 1960) kindly provided by the Department of Biological Standards, National Institute of Medical Research, London. The primary cell sheet was dispersed by trypsinization, the cell concentration adjusted to approximately 10^4 cells/ml. in growth medium, and distributed into tubes. In most cases semiconfluent growth was obtained within 48 hours.

Bottle cultures of M.S. (Tytell strain) (Kanda and Melnick, 1959), H.EpII (kindly provided by Dr. R. Chanock, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, U.S.A.), and HeLa (Tomlinson strain) were dispersed by trypsinization and dispensed into tubes at a concentration of 5×10^4 cells/ml.

Media

Growth medium for H.E.K. and for primary and secondary M.K. cultures consisted of Hanks's saline (with 0.35 g. of sodium bicarbonate per litre) 85%; heated calf serum, 5%; and lactalbumin hydrolysate, 10% of 5% stock solution in Hanks saline. In this and all other media penicillin 100 units, streptomycin, polymyxin B, and nystatin 100 µg./ml. were incorporated.

Standard maintenance medium (E.M. medium) consisted of Earles saline with 2.2 g. of bicarbonate per litre, 93%; plus 2% heated calf serum and 5% lactalbumin solution.

For experiments with common cold viruses the medium consisted of Hanks's saline plus 2% calf serum and 5% lactalbumin hydrolysate solution. In preliminary experiments the bicarbonate concentration of the medium was 0.35 g./l.; in later isolation and passage experiments this was increased to 0.52 g./l. In this latter medium ("C.C." medium), the appearance and survival time of tissue culture sheets was much better without any apparent loss of susceptibility to the standard H.G.P. strain of Salisbury viruses.

HeLa cultures were grown in Hanks's saline containing 20% calf serum and 10% lactalbumin solution; M.S. cells were grown in "199" medium (0.88 g. of bicarbonate (Glaxo) per litre) containing 10% calf serum, and H.EpII cells in M.K. growth medium.

Clinical Specimens

Nasal washings were obtained by instilling into each nostril 199 medium containing 1,000 units of antibiotics per ml. and getting the patient to snort out into a wide-mouthed sterile jar; 5–10 ml. of washings was collected from each case. After homogenization in a mechanical blender and centrifugation at 1,500 r.p.m. for 10 minutes the material was divided into small tubes and stored at –70° C. until used. Throat or nose swabs were

obtained on stick pledgets, broken off into 5 ml. of 199 medium, and homogenized and centrifuged as for nose washings before storing. Samples were taken on the first day of coryza and repeated 10 to 15 days after the symptoms had subsided.

Inoculation of Specimens

0.2–0.3 ml. of material was inoculated into each of 4 H.E.K. and M.K. tubes containing C.C. medium, and incubated at 33° C. with constant rolling ("C.C." conditions); the tubes were selected one to two days before full confluence. Additional inoculations were made into M.K. and H.E.K. tubes containing E.M. medium incubated without rolling at 37° C. ("routine diagnostic" conditions). All attempted isolations from clinical material were segregated strictly from passage experiments with known viruses. All tubes were observed daily for cytopathic changes, and at intervals of two to three days for haemagglutination or haemadsorption (Vogel and Shelokov, 1957) with human group O red cells. Tissue-culture fluids were changed as indicated by fall in pH; fluids harvested on the fourth to sixth day were used for passage to further cultures, but each tube was observed for 14 days before concluding that cytopathic effect was absent.

To guard to some extent against picking up "wild" agents from the tissues themselves on serial passage, fluid from uninoculated tubes of each batch of tissue employed has been passed serially through each subsequent batch. No cytopathic or haemadsorption agents have appeared as a result of this control manoeuvre.

Neutralization Tests

Initial 1/5 dilutions of all sera in 199 medium were inactivated at 56° C. for 30 minutes before further serial dilution in 199. Virus seed cultures were diluted where necessary in 199 to a concentration of 100 TCD₅₀ per 0.1 ml. Aliquots of virus and serum dilutions were incubated for 3 hours at 37° C., and 0.2 ml. of each mixture was then inoculated into each of four H.E.K. or M.K. tubes incubated under C.C. conditions. Tubes were scored daily for 14 days for cytopathic effect. Normal human or rabbit sera were included in each batch of tests.

Standardization of Methods

Before embarking on a study of the viral aetiology of natural common colds it seemed essential to investigate the sensitivity and selectivity of the present tissue-culture techniques, based on those used by Tyrrell and Parsons (1960).

Cultures of the prototype Salisbury virus H.G.P. and immune rabbit anti-H.G.P. serum were kindly provided by Dr. D. A. J. Tyrrell. The method of preparation of this antiserum will be described (Tyrrell, 1960); in end-point neutralization tests with 100 TCD₅₀ of virus per ml. a titre of approximately 1/200 was obtained.

Reproducible cytopathic effects, similar to those described by Tyrrell and Parsons (1960) were obtained on both M.K. and H.E.K. tissue cultures under C.C. conditions; no cytopathic change occurred in parallel cultures under "routine diagnostic" conditions. Serial passage of the cytopathic agent was successful through each batch of M.K. and H.E.K. tissue used in the subsequent experiments. Cytopathic effect began within three to five days of inoculation and proceeded from discrete well-demarcated virus plaques to complete cell-

sheet degeneration in four to five days; peak titres of virus in harvested tissue-culture fluid were approximately 10³ TCD₅₀/ml.

To determine whether these cultural conditions were equally satisfactory for primary isolations of "common cold" viruses from human material a small-scale volunteer experiment was performed.

Seven medical students, continuing to work in their normal environment, were given intranasal instillations of 0.5 ml. of a 1/10 dilution of a known H.G.P. virus stock (provided by Dr. Tyrrell); seven similar students were given non-infected material. This and subsequent human volunteer studies will be reported elsewhere. Two students developed typical coryza; both were in the virus-inoculated group. From nasal washings taken on the first day of illness (five days after inoculation) a cytopathic agent was isolated both on M.K. and H.E.K. tissue-cultures under C.C. conditions. No cytopathic change occurred in M.K. and H.E.K. under routine diagnostic conditions at 37° C. after inoculation either of original nasal washings or of positive culture fluids harvested from tubes incubated at 33° C. A repeated nasal lavage from these two volunteers after complete recovery from coryza did not contain demonstrable virus.

The two virus strains obtained were neutralized specifically by anti-H.G.P. rabbit serum: pre-infective sera from the two individuals (cases 47/60 and 53/60) did not neutralize either of the viruses or the standard H.G.P. strain, whereas convalescent serum samples neutralized both H.G.P. virus and the two isolates (H.G.P./47/60 and H.G.P./53/60) to similar titre.

The selectivity of C.C. conditions was investigated by parallel inoculation of M.K. and H.E.K. cultures under C.C. and routine diagnostic conditions with stock cultures of various known respiratory viruses in concentrations varying from 10⁻¹ to 10⁻⁴ dilutions of tissue-culture fluid pools. The test viruses included influenza A/Jap/305/EFME, parainfluenza 1 (Sendai), parainfluenza 2 (C.A. virus, Firth strain), parainfluenza 3 (H.A.1), R.E.O. type 1 (Echo type 10 prototype strain), adenovirus type 2, Coxsackie type B4, and herpes simplex virus. All tubes were examined daily for cytopathic effect, and in the case of the myxoviruses tubes showing no cytopathic change were examined by haemadsorption. In all cases similar virus replication occurred under both sets of cultural conditions.

These findings suggest that, although optimal cultural conditions for common-cold viruses involve lower temperatures and lower pH than for most respiratory viruses, infections caused by known myxoviruses, etc., are not likely to be missed on examining clinical specimens under C.C. conditions.

Investigations with Clinical Material

Twenty-five persons with typical coryza were investigated; 18 patients suffering from various mild upper respiratory tract febrile infections were similarly examined, and 8 normal people with no respiratory symptoms over the previous 10 to 20 days were also included. Nasal washings and throat swabs or sputum, where available, were inoculated into M.K. and H.E.K. tissue under C.C. and routine diagnostic conditions as described above. In most cases one or two blind passages were made from the fluids of negative primary inoculations, but in no case did this procedure

reveal cytopathic agents. The virus isolations from primary inoculations are summarized in Table I.

TABLE I.—*Virus Isolations from Nasal Lavage and Throat Swabs Under Various Cultural Conditions*

Type of Case	No. of Cases Tested	No. of Positive Isolations			
		Cultures at 33° C. C.C. Conditions		Cultures at 37° C. Routine Conditions	
		M.K.	H.E.K.	M.K.	H.E.K.
Coryza	25	1*	8	0	0
Infection of upper respiratory tract	18	2†	2	2	2
Healthy individuals	8	0	0	0	0

* This isolate (30/60) was also isolated on H.E.K. at 33° C.

† Adenovirus type 3 was isolated from two cases under all cultural conditions.

No cytopathic or haemadsorption agents were isolated from any of the nasal specimens from normal people. From the assorted respiratory infections adenovirus type 3 was isolated from two cases under both C.C. and routine diagnostic conditions; no other cytopathogenic or haemadsorption agents were found.

From 8 of the 25 persons with natural common colds, cytopathic agents were isolated on H.E.K. incubated under C.C. conditions; in only one of these (Case 30/60) was primary virus isolation also made on M.K. under C.C. conditions, and in no case did cytopathic changes occur in cultures under routine conditions at 37° C.

Duplicate nasal washings from 9 cases were transported in CO₂ ice to the Common Cold Research Unit, Salisbury. In blind tests, virus isolations were achieved in all five of the specimens found to be positive in this laboratory (Tyrrell, personal communication).

The properties of virus strains 30/60 and 16/60 have been investigated in more detail than other isolates, and these results will be described separately.

Virus 30/60

The patient was a 20-year-old female office worker. From nasal washings on the first day of coryza, virus was isolated both on H.E.K. and M.K. tissue under C.C. conditions and from throat swab on H.E.K. The virus was successfully reisolated from these sources after six months' storage at approximately -70°C.

The first virus generation derived from H.E.K. and that from M.K. could be propagated equally readily on either tissue under C.C. conditions with similar cytopathic changes, like those of H.G.P. virus; no haemagglutination or haemadsorption was detected. The fifth virus generation on either tissue remained equally capable of cross-propagation.

No cytopathic effect was obtained by inoculation of the first to fifth generations of virus into M.K. or H.E.K. at 37° C, either rolled or stationary, in either E.M. or C.C. medium.

Quantitative growth studies with 30/60 virus remain incomplete. Preliminary experiments, however, using cytopathic changes as the sole criterion, have helped to establish certain growth characteristics. In early passage experiments it was found that the greatest chance of successful propagation (and hence, presumably, peak virus titre) is from tissue-culture fluids harvested three to four days after inoculation, well before widely disseminated cytopathic change occurs. Fluids harvested after 50% of maximum cytopathic effect (six to nine days) have proved extremely unreliable for serial passage.

Infectivity titres of 30/60 virus propagated under C.C. conditions have remained low (approximately 10² TCD₅₀/ml.) up to the fifth virus generation.

There were no obvious differences in growth with bicarbonate concentrations varying from 0.35 to 0.88 g./l., calf serum concentrations from nil to 2%, or with change from Hanks-lactalbumin medium to 199. Cytopathic effects progressed uniformly from small discrete plaques to confluent degeneration only in tubes where the pH did not fall significantly below pH 7. At lower pH, where either the medium was left unchanged beyond five days or the bicarbonate concentration was reduced below 0.35 g./l., cytopathic changes ceased to advance; no secondary virus plaques appeared and the early focal lesions regressed.

In M.K. tubes incubated at 33° C. in C.C. medium no cytopathic change was observed over 14 days' stationary incubation after inoculation of 0.2 ml. of undiluted fluid of third-generation 30/60 virus. M.K. or H.E.K. cultures inoculated from 5 to 17 days after seeding approximately 10⁵ cells per tube showed similar cycles of cytopathic change, suggesting that the age of the cell sheet is not critical for susceptibility to 30/60 virus; propagation on primary M.K. cultures has been as reproducible as with secondary cultures. H.E.K. cultures derived from embryos 12-28 weeks old proved equally susceptible to 30/60 virus, but one batch of kidney tissue from a 3-day-old child produced no cytopathic change with this virus or with strains 16/60, 1/60, or 20/60; fluids harvested from these cultures did not produce cytopathic changes on passage to further H.E.K. or M.K. batches.

The cell sheets of this baby kidney were perfectly normal in all growth characteristics, and primary isolations of adenoviruses were made on this tissue. In similar experiments with H.G.P. virus (Flewett, personal communication) no cytopathic effect occurred on human neonatal kidney tissue, though concurrent inoculations of M.K. and H.E.K. tubes demonstrated normal virus growth.

The biological characteristics of 30/60 virus appear superficially similar to those of H.G.P. virus, but cross-neutralization experiments (Table II) suggest that the

TABLE II.—*Cross-neutralization Tests on H.E.K. Cultures with Viruses Isolated from Coryza*

Virus Strain	Rabbit Anti-H.G.P. Serum	Paired Sera from Cases of Coryza						Rabbit Anti-16/60 Serum	Normal Rabbit Serum
		53/60		30/60		16/60			
		A	C	A	C	A	C		
H.G.P.	+	0	+	-	-	-	-	-	0
H.G.P./53/60	+	0	+	0	0	0	0	0	0
30/60	0	0	0	0	+	0	0	0	0
16/60	-	0	0	-	0	0	+	+	0

+ = Neutralization of virus at 1/10 serum dilution.

0 = No neutralization.

- = Untested.

A = Acute illness serum sample.

C = Convalescent serum sample.

¹ In subsequent titration, neutralization titre = 1/40.

² In subsequent titration, neutralization titre = 1/20.

³ In subsequent titration, neutralization titre = 1/80.

two viruses are serologically different from each other and from viruses isolated only on H.E.K.

Virus 16/60

The patient was a female house-physician in another Sheffield hospital; there was no contact with the virus laboratory. From nasal lavage on the first day of coryza a cytopathic agent was isolated on H.E.K. under

C.C. conditions. After six virus generations through H.E.K. cytopathic effects regularly began within 48 to 72 hours after inoculation and proceeded to full degeneration by six to seven days. As with 30/60 virus, efficiency of passage decreased sharply in culture fluids harvested beyond the 50% level of cytopathic change. The peak titre of the sixth virus generation was approximately 10^4 TCD₅₀/ml.; cytopathic change occurred only on incubation at 33° C. in low bicarbonate medium, but rolling did not seem to be essential.

No cytopathic agent was isolated from the nasal washings on primary inoculation of M.K. tubes under C.C. conditions nor on two subsequent blind passages.

The third virus generation was inoculated into suckling mice intracerebrally and subcutaneously and into adult mice and weanling hamsters intranasally without obvious pathological effect.

In neutralization tests with 100 TCD₅₀ of fourth-generation 16/60 virus per 0.1 ml. (Table II) a rise in antibody titre from <1 in 5 to 1 in 20 was demonstrated with paired sera from Case 16/60. No inhibition occurred with two samples of normal human serum or normal rabbit serum. There was no neutralization by animal or human anti-H.G.P. sera; conversely, convalescent serum of Case 16/60 at 1 in 10 dilution failed to neutralize H.G.P. or 30/60 virus.

An immune antiserum against 16/60 virus was prepared in rabbits by a multiple-depot-antigen technique (Hayward and Augustin, 1957). The antigenic material was obtained by fluorocarbon purification (Epstein, 1958) of 200 ml. of tissue-culture fluid from the fourth H.E.K. generation, concentrated tenfold in the Spinco L model centrifuge at 40,000 r.p.m. for 45 minutes.

The antiserum had no cytotoxic effect on H.E.K. A neutralization titre of 1 in 80 was detected with homologous 16/60 virus, but the serum did not inhibit growth of 30/60 or H.G.P./53/60 virus.

Other Viruses

All these strains were isolated on H.E.K. only, and, though all have been passed successfully through four generations on this tissue under C.C. conditions, titres have remained low. No cytopathic change was observed after inoculation of M.K. at 33° or 37° C. or in H.E.K. at 37° C. with first to third generations of any of these strains. Preliminary neutralization spot-tests showed that in three cases (1/60, 20/60, and 119/60) antibody to the homologous virus was present at 1 in 10 dilution in the convalescent-serum sample. These sera did not neutralize 16/60 virus, but convalescent serum of Case 16/60 contained neutralizing antibody for viruses 1/60, 20/60, and 119/60.

The difficulty in obtaining supplies of H.E.K. tissue large enough for multiple neutralization-titration tests has delayed further attempts to establish the relationship of these viruses to each other, to virus 16/60, or to F.E.B., the prototype Salisbury H.E.K. virus.

Growth of Common-cold Viruses on Continued Cell Line

Fourth H.E.K. generation virus pools of H.G.P./53/60, 30/60, and 16/60 failed to produce cytopathic effects on tube cultures of M.S. or HeLa cells incubated under either C.C. conditions or routine diagnostic conditions. Further investigations are in progress to determine

whether inapparent—that is, non-cytopathic—virus growth occurs in these cell lines

In tube cultures of H.EpII cells, however, inoculation of H.G.P./53/60 resulted in cytopathic changes (see Fig.) closely similar to those seen in M.K. cultures. Under

Cultures of H.EpII cells maintained at 33° C. in C.C. medium with 0.52% bicarbonate. Above: Normal uninoculated tube—occasional small round cells in mitosis can be seen. Below: Three days after inoculation of virus H.G.P./53/60. Large irregular refractile cells can be seen. (Unstained unfixed preparation. $\times 100$.)

routine diagnostic conditions these changes were inconstant and non-progressive, only a few small scattered foci appearing at the edges of the cell sheet. Under C.C. conditions microplaques were visible within 12 hours of inoculating 10^2 (H.E.K.) TCD₅₀ of virus, and rapidly progressed to complete cell-sheet degeneration. Tissue-culture fluids harvested at 50–75% cytopathic change have undergone successful passage through four generations under C.C. conditions. The third H.EpII virus generation pool had a titre of approximately 10^5 TCD₅₀/ml. when titrated on further H.EpII cultures, and a 10^{-1} dilution of the pool was specifically neutralized by anti-H.G.P. antiserum. On

M.K. cultures this H.EpII-derived pool produced similar titres under C.C. conditions only, with typical cytopathic changes of H.G.P. virus; again growth was specifically neutralized by anti-H.G.P. antiserum.

Strain H.G.P./53/60 was successfully reisolated on H.EpII cultures from the original nasal washings after storage at -70°C . for six months.

Similarly, virus 30/60 in the fourth H.E.K. generation gave characteristic cytopathic effects on first passage to H.EpII under C.C. conditions; the harvested tissue-culture fluid produced cytopathic effects on both H.EpII and M.K. cultures under C.C. conditions, but no visible effect occurred under routine diagnostic conditions. The titre of H.EpII-derived 30/60 virus appeared to be less than 10^2 TCD₅₀/ml. when estimated on either M.K. or H.EpII cultures, and the development of cytopathic effect was much slower than with H.C.P./53/60 on the same tissue.

No cytopathic effect on H.EpII cells has been observed after inoculation of 10^3 (H.E.K.)TCD₅₀ of 16/60 virus or on two serial blind passages made from the negative primary tubes. A seed pool of F.E.B. virus (kindly provided by Dr. Tyrrell) has also failed to give cytopathic effects in H.EpII cultures. The possibility of inapparent virus growth under these conditions is being investigated.

Discussion

Tissue cultures of M.K. or H.E.K. incubated at 33°C . in low bicarbonate medium with constant rotation allow the cytopathic effects of the Salisbury group of viruses to be demonstrated readily. A range of known respiratory viruses have been shown to grow as well under these circumstances as in conventional stationary cultures at 37°C . in alkaline medium.

In the present experiments eight virus strains were isolated from 25 cases of natural coryza under C.C. conditions of incubation only, and subsequent generations of these agents have failed to produce cytopathic changes under routine diagnostic conditions at 37°C . These viruses were closely similar in cytopathic effect and in growth characteristics to the prototype Salisbury strains described by Tyrrell and Parsons (1960). Strain 30/60 resembled the Salisbury strain H.G.P. in its ability to produce visible effects both on M.K. and on H.E.K. tissue, but is clearly distinguished from H.G.P. virus on serological grounds. The other seven isolations made in this laboratory closely resemble the Salisbury strain F.E.B. in their limitation to H.E.K. cultures, their cytopathic changes, and general biological properties. Virus F.E.B. was deliberately excluded from this laboratory until the present series of experiments were completed, and, as no immune anti-F.E.B. serum was available, there has yet been no opportunity of confirming the relationship of our isolates to this prototype Salisbury strain. It seems possible that the seven virus strains described may be related to each other, but they are quite distinct serologically from either the 30/60 or the H.G.P. strains which grow on M.K.-tissue culture. It is of interest that both M.K. viruses (H.G.P. and 30/60) can be propagated readily on H.EpII tissue cultures under C.C. conditions, whereas the H.E.K. viruses F.E.B. and 16/60 fail to produce cytopathic effect.

It cannot be said from these preliminary experiments that Koch's postulates are completely fulfilled, or that the agents are truly common-cold viruses. However, each of the viruses was isolated from a case with coryza

only during the "cold," and nasal washings obtained after recovery were negative. No similar viruses were isolated from uninoculated-tissue-fluid passages, or from inoculations from normal individuals or cases of mild infection of the respiratory tract other than coryza. In most patients from whom a virus was isolated, neutralizing substances appeared in the convalescent serum. In two cases viruses reisolated in Salisbury from nasal washings obtained here (strains 16/60 and 30/60) have been shown in human volunteer experiments to produce an experimental coryza like that induced by H.G.P. virus (Tyrrell, personal communication).

These early results are encouraging in that they suggest that many of the findings of the Salisbury group of workers are relevant to a study of natural common colds. Further work in this laboratory will be directed to an investigation of the distribution of "cold" viruses in the general population, both by cultural and by serological means, and to the study of their aetiological role in coryza or other respiratory infections.

Summary

The effect produced by the H.G.P. strain of Salisbury viruses under low temperature and low bicarbonate conditions has been confirmed on monkey-kidney and human-embryo-kidney tissue cultures.

From 25 cases of natural coryza in Sheffield, eight virus agents with similar environmental requirements have been isolated. A rise in homologous neutralizing antibody was found in paired sera from five of the positive cases. Only one of these eight agents resembles the Salisbury H.G.P. strain in its ability to give cytopathic change on both M.K. and H.E.K. tissue culture. This virus strain, 30/60, is, however, serologically distinct from H.G.P.

Seven of the eight Sheffield isolates resembles the Salisbury strain F.E.B. in that isolation and passage were possible only on human-embryo-tissue culture. These agents are serologically distinct from the viruses capable of growth on monkey kidney, but may be serologically related to each other. Tissue cultures of the continuous cell line H.EpII support the cytopathic growth of H.G.P. and 30/60 viruses, and primary isolation has been made on this tissue. The group of viruses incapable of growth on monkey kidney failed to give cytopathic effect on H.EpII cells.

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The report on the one-day conference held on December 18, 1959, at the Royal College of Nursing on the subject of "The Employment Problems of the Home-bound Disabled" has now been published. Copies of this may be obtained from the British Council for Rehabilitation of the Disabled, price 2s. 10d. (including postage).