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compared with the incidence of 12.6% in a series of 2,352 cases collected by the writer from the literature of the earlier period in the history of pneumothorax, when the precautions detailed above were not usually observed. Obviously empyema will continue to occur when pneumothorax is used, in spite of such precautions, but the incidence may be lowered by these precautions. Empyema occurs almost entirely in the first three years of pneumothorax treatment. It seems obvious that it is a complication of active stages of tuberculosis.

In this series, chemotherapy was used only late in the course of cases which had relapsed or required major surgery to achieve arrest. It seems reasonable to expect that, if chemotherapy by modern methods is started as soon after diagnosis as possible, pneumothorax will be even more successful.

A study of the literature was made by the writer[1] on the effectiveness of pneumoperitoneum compared with that of pneumothorax as a collapse measure, estimated objectively by the criteria of sputum conversion and cavity closure. This study showed pneumothorax to be clearly superior to pneumoperitoneum as a method of collapse therapy. If chemotherapy can make pneumothorax safer, as seems probable, the method of treatment described in this article as "primary pneumothorax treatment" will give results that are likely to be very satisfactory. The inferior collapse method, pneumoperitoneum, will not have such a great attraction.

Thoracoplasty, with its deformity, is in itself not infrequently a cause of disability and, like resection, is inseparable from the occasional fatality.

Resection of a localized lesion *after* reaching the "target point" of arrest is another subject not to be discussed here.

There is no doubt in the mind of the writer that chemotherapy started early will make any form of collapse therapy unnecessary for many cases. It seems probable that pneumothorax will return to popularity under the protection of chemotherapy as the most universally applicable method of collapse therapy for those cases not brought to arrest by chemotherapy and bed rest alone. The therapeutic use of posture while at rest in bed should not be forgotten.

SUMMARY

A series of 499 cases of pulmonary tuberculosis in which pneumothorax was induced or attempted as the primary method of treatment was followed up for five years from the induction or attempt, and a survival rate of 91.3% for the whole series was found. The incidence of empyema was 7.1%. The fatality rate in the whole series among empyema cases was 1.4%.

This result is considered good. The same approach, together with early use of chemotherapy, is considered likely to be the method of choice in the future.

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RAPID DIAGNOSIS OF HERPETIC INFECTIONS BY ISOLATION OF VIRUS IN TISSUE CULTURES*

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RECENT IMPROVEMENTS in the techniques of tissue culture have led to the introduction of methods for the rapid diagnosis of several virus infections, for example, poliomyelitis.[1]

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The laboratory diagnosis of infections caused by the virus of herpes simplex has been made until recently by inoculation of animals or chick embryos. The cornea of the rabbit is susceptible to infection with herpes virus, and a typical ulcerative keratitis develops. The virus kills suckling mice, and produces characteristic plaques on the chorioallantoic membrane of the chick embryo. The main disadvantage of these methods is that a final report cannot be issued promptly to the clinician. Infection occurs within 24-48 hours, but some days are required for the histological examination on which the final diagnosis is based.

Scott *et al.*[2] have shown that tissue cultures of rabbit cornea are susceptible to herpes virus, and

may present histological evidence of infection within a few hours. The present paper describes the application of a similar method to the rapid laboratory diagnosis of herpetic infections.

MATERIALS AND METHODS

Cases studied

Laboratory investigations have been carried out on 16 patients, nine with keratitis and seven with stomatitis.

Preparation and maintenance of tissue culture

Thin slices of epithelium were shaved from the cornea of freshly killed rabbits, and were chopped into fragments approximately 1 mm. in diameter. Three fragments were placed directly on the surface of a coverslip (11 x 40 mm.) already kept at 37° C. for several hours. Each coverslip was inserted into a pyrex culture tube, which was refrigerated at 4° C. for one hour.[3] A quantity of 3 ml. of "natural feeding mixture" was then added. This medium consisted of Hanks' balanced salt solution, 3 parts, and ox-serum ultrafiltrate, one part, with the addition of 2.5% beef embryo extract, 5% normal horse serum, and 100 units of penicillin and 100 micrograms of streptomycin per ml. The cultures were then placed horizontally in a rack in a 37° C. incubator, and were left undisturbed for three or four days.

At this time, a good growth of epithelial cells was usually present around the fragments (Fig. 1). The feeding mixture was then replaced with Medium No. 199 with 2.5% horse serum and antibiotics.[4] The inoculum to be tested was added at this stage, and the cultures were then placed in a "roller tube" drum and rotated in a 37° C. incubator.

Inoculation of tissue cultures

Specimens consisted of washings and scrapings from the conjunctival sac of patients with keratitis, and mouth washings from persons suffering from stomatitis. Penicillin (1,000 units per ml.) and streptomycin (200 micrograms per ml.) were added, and the specimens were left for 30 minutes at room temperatures. They were then inoculated in each of four cultures (0.25 ml. each). Inoculated cultures were rotated at 37° C., and were examined microscopically at frequent intervals.

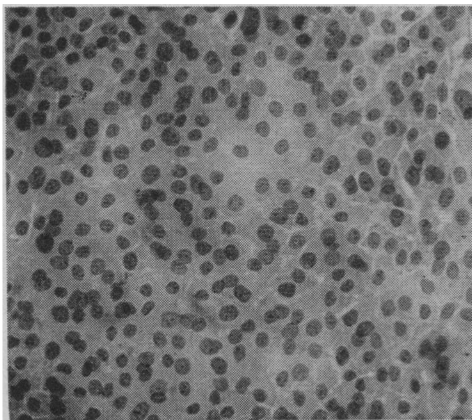

Fig. 1

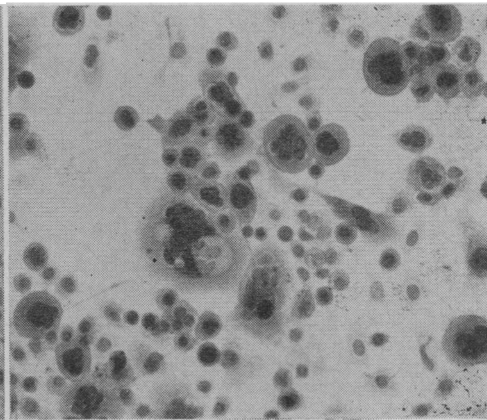

Fig. 2

Fig. 1.—Normal rabbit corneal epithelium; 4 days' growth in tissue culture. Hæmatoxylin and eosin. × 154. Fig. 2.—Rabbit corneal tissue culture 20 hours after inoculation with saliva from case of stomatitis. Note general cytopathogenic effect and presence of giant cells. Hæmatoxylin and eosin. × 154.

Experiments with a laboratory-passed strain of herpes simplex virus were also carried out. This strain (H51) was isolated in 1951 on the cornea of a rabbit inoculated with material from a patient showing central corneal ulceration. This strain has been maintained recently by passage in tissue cultures of rabbit cornea.

Histological examination of tissue cultures

Coverslip preparations were fixed in a solution of methyl alcohol (94 c.c.), formalin (5 c.c.) and acetic acid (1 c.c.). These preparations were then stained by hæmatoxylin and eosin. The entire histological procedure could be carried out in less than two hours.

RESULTS

Changes observed in infected tissue cultures

In order to learn how early herpetic infection can be recognized in rabbit corneal tissue cultures, experiments were first carried out with the stock strain of virus (H51). Coverslip cultures were stained at two-hourly intervals following infection. The earliest sign of infection was the appearance of intranuclear inclusions after six hours. These inclusions were eosinophilic, and were surrounded by an unstained halo. Some infected nuclei showed a margination of the chromatin on the nuclear membrane.

By the tenth hour many infected cells contained nuclear inclusions of a somewhat different appearance. These inclusions filled the entire nucleus and stained pale blue or purplish. In some instances there were scattered clumps of chromatin distributed over the surface of the inclusions.

For about 12 hours following infection the epithelial sheet appeared relatively intact. Soon after this, degenerative changes began to appear, with the formation of clumps of infected cells which retained eosin more intensely. At about this time characteristic giant cells were present. These cells closely resembled the "Tzanck cells" found in smears from the base of vesicles of herpes simplex.[5] As infection proceeded there was a corresponding loss of cells.

Preparations examined in the wet state usually did not show recognizable signs of infection until 12 hours. After this time, infection was suggested by the presence of focal areas of rounded, refractile cells.

The series of cultures inoculated with pathological specimens were left overnight before staining, although it was confirmed that changes occurred as early as after 6-8 hours. Infection was recognized by the finding of intranuclear inclusions of both types mentioned, widespread degeneration of epithelium, and the formation of numerous giant cells (Figs. 2-4). It was found that some specimens produced characteristic changes more rapidly than others.

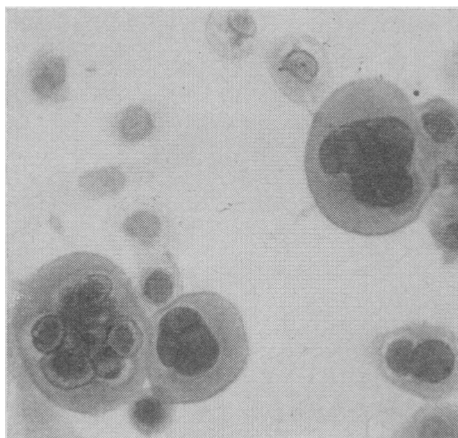

Fig. 3

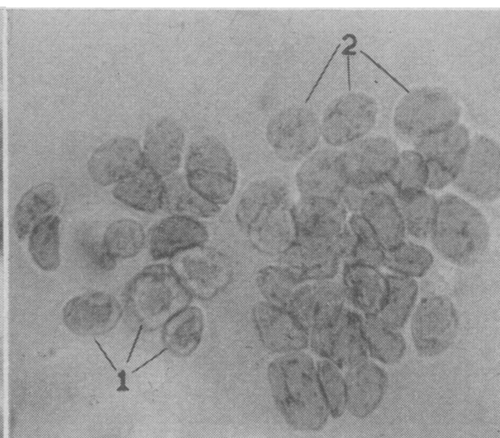

Fig. 4

Fig. 3.—Enlargement of giant cells shown in Fig. 2. Note Type A inclusions in multinucleate cell at left. Hæmatoxylin and eosin. × 408. Fig. 4.—A group of inclusion-filled nuclei in rabbit corneal tissue culture 20 hours after inoculation with saliva from case of stomatitis. Note distribution of chromatin. (1) Type A inclusion, with halo. (2) Large type of inclusion without halo. Hæmatoxylin. × 576.

The changes that we have found appear to be similar to those described by Scott *et al.*[2]

More recently, experiments have been carried out with other types of tissue culture which have become available to us; these include HeLa carcinoma cells and human kidney cells (trypsin-treated). Scherer and Syverton[6] have shown that HeLa cell cultures are susceptible to herpes simplex, and have described both Type A inclusions and multinucleated cells. We have found it convenient to use both HeLa cell cultures and human kidney cell cultures in our investigation of herpetic infections, and these techniques are recommended to laboratories with the necessary facilities for their preparation and maintenance. Rabbit corneal tissue cultures are simpler to prepare and the tissue is more generally available.

Sensitivity of diagnostic methods

Of the 16 patients with suspected herpetic infection which were investigated, washings

from six with stomatitis and four with keratitis produced typical changes in tissue cultures; similar changes occurred on each of four successive passages in tissue cultures. Passage of nine of the ten tissue culture fluids to rabbits and suckling mice confirmed the presence of herpes simplex virus.

TABLE I.

LABORATORY FINDINGS IN CASES OF SUSPECTED HERPETIC INFECTION

Clinical diagnosis	*Case reference number*	*Presence of changes characteristic of herpes simplex*	
		In tissue cultures of rabbit cornea	*In rabbit cornea in vivo*
Stomatitis	2, 10, 14	+	+
	22	+	—
	12, 13	+	NT*
	7	—	—
Keratitis	19	+	+
	16, 20	+	—
	9	+	NT
	11, 15, 17	—	—
	6, 8	—	NT

*NT—Not tested.

In order to compare the sensitivity of tissue culture with that of animal inoculation, 11 of the 16 specimens were tested in parallel in tissue cultures of rabbit cornea and on the scarified cornea of a rabbit. From Table I, it will be seen that in no instance were signs of infection evident in the rabbit eye without a corresponding change in tissue cultures. However, material from three patients produced characteristic changes in tissue cultures, but not in rabbits.

DISCUSSION

The results of this study provide further evidence for the value of tissue culture techniques in the early laboratory diagnosis of virus infections. Detection of the causative agent is now possible in herpetic infection within eight to twenty hours of inoculation of the tissue cultures. This test is of considerable value in the differential diagnosis of conditions which may simulate herpetic infections; for example, herpangina of the throat caused by Coxsackie virus (Group A), skin lesions caused by herpes zoster and varicella viruses, and Kaposi's varicelliform eruption.

Because of the ready availability and moderate cost of rabbit tissue, the technique which we have described is within the capabilities of smaller laboratories engaged in virus research or diagnostic work. Equally satisfactory results can be obtained by the use of HeLa cultures or human kidney trypsin-treated cultures.

SUMMARY

1. Tissue cultures of rabbit corneal epithelium were used in the laboratory diagnosis of sixteen cases of presumed herpetic infections.

2. Washings from ten of these cases were found to contain herpes simplex virus by such inoculation.

3. Passage of nine of the ten culture fluids to rabbits and suckling mice confirmed the presence of herpes simplex virus.

4. Diagnosis was based on the presence of characteristic intranuclear inclusions and giant cells, and could be made within eight hours.

5. It appears that tissue cultures of rabbit cornea are at least as sensitive as the inoculation of the rabbit cornea *in vivo* for detection of herpes simplex virus in specimens obtained from patients suffering from keratitis and stomatitis.

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RÉSUMÉ

Le diagnostic de laboratoire dans 16 cas d'infection herpétique fut obtenu grâce à l'emploi de cultures de tissu épithélial de cornée de lapin. Il fut prouvé grâce à ces inoculations que les lavages de 10 de ces cas contenaient le virus de l'herpès. Le passage de 9 de ces 10 liquides de culture à des lapins et à de jeunes souris confirma la présence du virus. La démonstration des inclusions intra-nucléaires ainsi que des cellules géantes servit de base au diagnostic. Celui-ci peut se faire dans les 8 heures qui suivent l'inoculation de cultures de tissu. Il semble que la cornée du lapin comme milieu de cultures de tissu soit aussi sensible pour la recherche du virus herpétique que cette même cornée inoculée chez l'animal vivant pour ce qui est des spécimens obtenus de malades atteints de kératite et de stomatite.

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