absence of lesions in the olfactory bulbs as determined by serial sections. The oropharyngeal and gastrointestinal routes can also, in all probability, be considered as excluded on the basis of general experience with the rhesus monkey extending over more than thirty years during which it has been the standard test animal in poliomyelitis research, showing that animals of that species can only be infected non-traumatically by the olfactory route. Several extensive studies, of which those of Clark, Roberts and Preston[5] and of Flexner[6] may be cited, testify to the uniformly negative results of feeding virus to rhesus. It therefore seems highly probable that in the positive inhalation experiments with rhesus here reported, infection entered through the lower respiratory mucosa, at or below the epiglottis. The character of the symptoms in the two positive cynomolgus experiments with olfactory blockade, on the other hand, suggest entry through the oropharynx.

Further work along the same lines is in progress.

SUMMARY

Infection has been obtained in both rhesus and cynomolgus monkeys by inhalation of poliomyelitis virus in the form of droplet nuclei. The olfactory route was excluded in part of the animals successfully infected. The gastrointestinal route is believed to have been excluded in the rhesus monkeys. It seems most probable that the portals of entry were the lower respiratory mucosa in the case of the rhesus monkeys and the oropharyngeal mucosa in the case of the cynomolgus monkeys. Fever and occasional mild symptoms in 8 other rhesus monkeys suggest that an abortive form of poliomyelitis may have resulted from inhalation, but this can not at present be considered as proved. The experiments open up the possibility that human poliomyelitis may, at least sometimes, be an air-borne infection and that the lungs may be a portal of entry. Neither of these aspects of the disease has hitherto, so far as we are aware, been studied experimentally. The presence of virus in the human nasopharynx, which has been repeatedly demonstrated,[7, 8] provides an obvious source of air contamination by patients and carriers; and direct contact has been traced during epidemics in a considerable fraction of cases,[9] amounting to about one third in the report of Top and Vaughan.[10] The relative importance of transmission by air and by ingested material remains to be determined. It would seem probable, however, that both modes of infection must be taken into account.

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INHIBITORY EFFECTS OF SULFONAMIDES ON CULTURES OF ACTINOMYCES HOMINIS[1]

PRACTICALLY no information is available on the possible effects of *in vitro* sulfonamides on fungi, although there have been several reports of clinical benefits from the use of sulfonamide drugs in fungous infections.[2] This has resulted in an uncertainty as to whether the effect of the sulfonamides is on the fungi themselves or on secondary bacterial invaders. This paper reports the demonstration of direct inhibitory effects of sulfonamide drugs on cultures of *Actinomyces hominis*.

A stock strain (S) of *Actinomyces hominis* was cultured on Krainsky's glucose agar, and thioglycollate medium.[3] Another strain (M) was isolated on the thioglycollate medium from a draining sinus from the jaw of a patient and thereafter grown on veal agar medium. It differed from the former strain in that the organisms were shorter, seldom branched and often clubbed. The following drugs and concentrations were used: sulfanilamide, sulfathiazole and sulfadiazine,[3] in concentrations of 10, 50 and 100 mg per cent. incorporated in the media before autoclaving. The results obtained may be summarized according to aerobic and anaerobic conditions.

AEROBIC CONDITIONS

The S strain grown aerobically on Krainsky's medium was delayed in growth by concentrations of sulfanilamide of 10 and 50 mg per cent. but at the end of one month the growth in both concentrations equalled that of the controls (10 cultures). A concentration of 100 mg per cent., however, completely inhibited growth in some instances and allowed only slight growth in others by the end of one month (7 cultures). Sulfathiazole allowed moderate growth in 10 mg per cent. concentration, slight growth in 50 mg per cent. concentration, and no growth in 100 mg per cent. concentration (9 cultures). The observations in each case extended over one month. The results with

[5] P. F. Clark, D. J. Roberts and W. S. Preston, *Jour. Prev. Med.*, 6: 47, 1932.

[6] S. Flexner, *Jour. Exp. Med.*, 63: 157, 1936.

[7] S. D. Kramer, B. Hoskwith and L. H. Grossman, *Jour. Exp. Med.*, 69: 49, 1939.

[8] A. B. Sabin and R. Ward, *Jour. Exp. Med.*, 73: 771, 1941.

[9] Survey by International Committee for the Study of Infantile Paralysis Organized by Jeremiah Milbank. Williams and Wilkins Company, 1932, pp. 370 *et seq.*

[10] F. H. Top and H. F. Vaughan, Special Report of the Detroit Dept. of Health on Epidemiology of Poliomyelitis in Detroit in 1939.

[1] Aided, in part, by the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

[2] L. G. Dobson, E. F. Holman and W. C. Cutting, *Jour. Am. Med. Assoc.*, 116: 272, 1941.

[3] We wish to thank the Baltimore Biological Laboratory for supplies of thioglycollate medium, and the Lederle Laboratories for sulfadiazine.

sulfadiazine were similar to those with sulfathiazole (9 cultures).

The S strain was grown on thioglycollate medium for 20 days, at which time there was luxuriant growth in every tube. Autoclaved thioglycollate medium containing sulfanilamide, sulfathiazole or sulfadiazine was then added to the cultures in amounts to make the total drug concentration 50 or 100 mg per cent. (5 results with each drug in each concentration). Fifteen days later the tubes were subcultured. While the controls grew profusely, only 3 cultures from the sulfonamide groups showed any growth at all, and this was minimal. There was no growth in the subcultures of the sulfathiazole or the sulfadiazine tubes.

The M strain, when grown aerobically, gave a plentiful growth in one month. A similar amount of growth, although somewhat slower in development, occurred in cultures containing 10 mg per cent. of sulfanilamide (3 cultures). No growth occurred in cultures containing 50 or 100 mg per cent. sulfanilamide, or 10, 50 or 100 mg per cent. of sulfathiazole or sulfadiazine (3 results with each drug in each concentration).

Thus it is apparent that any of the 3 sulfonamide drugs used more or less completely inhibited the aerobic growth of both strains of actinomyces. Low concentrations were less effective than high, and sulfanilamide was less effective than sulfathiazole or sulfadiazine.

Anaerobic Conditions

The S strain was grown anaerobically on Krainsky's medium in sulfanilamide, sulfathiazole and sulfadiazine, at 10, 50 and 100 mg per cent. concentrations (3 results with each drug in each concentration). Growth was plentiful in the control tubes, and in one of the sulfanilamide (10 mg per cent.) tubes in one month. Of the remaining 26 tubes, 8 showed very slight growth, while 18 showed no growth. The positive growths occurred irregularly in the presence of the various drugs in different concentrations.

The S strain was grown anaerobically on thioglycollate medium with sulfanilamide, sulfathiazole and sulfadiazine in concentrations of 10, 50 and 100 mg per cent. (3 results with each drug in each concentration). In comparison with the vigorous growth in the control tubes in one month, sulfanilamide permitted equally good growth in 10 and 50 mg per cent. concentrations, and poor or no growth in 100 mg per cent. concentrations. Sulfathiazole allowed fair growth in 10 mg per cent. concentrations, poor or none in 50 and 100 mg per cent. concentrations. Sulfadiazine allowed little or no growth at any concentration.

The M strain was grown anaerobically with sulfanilamide, sulfathiazole and sulfadiazine in concentrations of 10, 50 and 100 mg per cent. (3 results with each drug in each concentration). Growth in the control tubes was moderate, and that in sulfanilamide (10 mg per cent. concentrations) was almost as good, but it was absent in all other tubes after one month.

The anaerobic results, therefore, agreed with the aerobic.

Conclusions

(1) Aerobic and anaerobic cultures of two strains of *Actinomyces hominis* were inhibited to some extent by sulfanilamide in a concentration of 10 mg per cent.

(2) Concentrations of 50 and 100 mg per cent. checked growth more or less completely.

(3) Sulfathiazole and sulfadiazine were definitely more effective than sulfanilamide in similar concentrations.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN APPARATUS FOR ROLLER TUBE TISSUE CULTURE

THE roller tube method of tissue culture, originated by Gey[1] and developed by Lewis,[2] is being more and more widely adopted. The apparatus usually employed, consisting of a 1/20 h.p. electric motor, reduction gears and a rotating tube carrier, has several disadvantages. It is expensive and causes more or less noise and vibration. The motor can not be housed inside the incubator because it develops sufficient heat of itself to exceed 38° C. This necessitates a cumbersome apparatus with a shaft running from the motor through the wall of the incubator to the tube carrier.

A relatively simple and inexpensive device which does not have these disadvantages has been successfully used in this laboratory. The apparatus consists of a motor and tube carrier conveniently mounted to form one complete unit.

The general structure of this apparatus is shown in Fig. 1. The motor, A, was obtained from a General Electric clock, of the dressing table type, having an automatic starting mechanism. The motor was detached from the clock and removed from its metal housing. The exposed gears were removed until there

[1] G. O. Gey, *Am. Jour. Cancer*, Vol. 17, 1933.
[2] Warren H. Lewis, Contributions to Embryology No. 150, July, 1935.