To be sure that all of the oscillations recorded come from the extended finger several control tests were made. With either a constant pressure or with no pressure on the platform of our generator no oscillations were detected. With either the input wires of the amplifier short circuited or with them open no disturbances of any kind appeared in the records. Great care had to be taken to eliminate the effects of tones and noises. However, we feel certain that mechanical, acoustical and electrical artifacts do not appear in our recordings.

There remains the problem of allocating the possible source of these tremor frequencies.

5872

## Cultivation of the Virus of Common Cold and Its Inoculation in Human Subjects.

H. M. POWELL AND G. H. A. CLOWES.

*From the Lilly Research Laboratories, Indianapolis.*

In a series of papers Dochez[1] and his associates have presented evidence for the virus etiology of the common cold and have described experiments on the cultivation of this virus in tissue media and its inoculation into human subjects and chimpanzees. The purpose of this investigation has been to cultivate the virus of common cold and during the period of the year when the incidence of common colds is low to conduct human inoculation experiments on volunteers not subject to isolation.

The strain of common cold virus employed in these experiments was derived from a patient, "A. R.," suffering from a cold of more than usual severity. Nasal washings were obtained on March 26th, 1931, within the first 24 hours of the onset of the cold. Ten cc. of Tyrode solution were flushed through each nostril and expelled through the mouth. These washings were promptly passed through a Seitz filter and cysteine hydrochloride added to a concentration of 1:2000. The filtered washings were introduced in 1 cc. amounts

[1] Dochez, A. R., Shibley, G. S., and Mills, K. C., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 562; **27**, 59; *J. Exp. Med.*, 1930, **52**, 701. Shibley, G. S., Mills, K. C., and Dochez, A. R., *J. Am. Med. Assn.*, 1930, **95**, 1553. Dochez, A. R., Mills, K. C., and Kneeland, Y., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513; **29**, 64.

into 9 cc. hashed chick embryo tissue culture medium containing cysteine hydrochloride in a concentration of 1:2000. The culture was incubated under vaseline seal at 37°C. for 4 days. A Seitz filtrate was prepared from this first culture generation and stored under vaseline seal at 4°C. Further tissue cultures and filtrates were prepared in series as described above, the periods of incubation being from 4 to 5 days and the intermediate periods of ice-box storage of the Seitz filtrate averaging 5 to 6 days. At the present time this cold virus is in the 31st generation and has been cultivated successfully outside the body for a period of 8 months.

Attempts to induce common colds by inoculation into human volunteer subjects were carried out as follows:

About 1 cc. of virus filtrate was instilled into each nostril as the subject lay upon his back. The head was turned toward one side and then toward the other. After a few minutes the instillation was repeated and the subject turned over upon his face to allow of more adequate wetting of the membranes. This procedure was usually carried out in the morning and repeated about 5 or 6 hours later in a routine fashion.

These test subjects were not isolated but were selected from large groups of comparable individuals working under the same conditions in a large manufacturing unit. The experiments were conducted during the spring and summer when the incidence of colds was low in the group of workers and their families from whom the test individuals were selected. Only those individuals were subjected to test who had not suffered from colds within 2 months of the date of the experiment but were known to average at least 2 or 3 colds each winter, which would indicate that they were susceptible to colds. Cultures were made for 2 days prior to inoculation and all individuals showing hemolytic streptococci and pneumococci of fixed types were excluded from test.

Before and during each such inoculation experiment the incidence of common colds was ascertained in the control group consisting of individuals working in the same environment and under the same conditions as those subjected to the test. Transmission experiments were not started at any time when the incidence of colds in the control group proved to be more than 10% during the week preceding the experiment.

Table I gives a record of the cultivation of this strain of common cold virus and the results of inoculation experiments on presumably susceptible human test subjects. It will be seen that from 60 to 80% of the subjects used experimentally contracted these in-

TABLE I.
*"A.R." Strain of Common Cold Virus: Human Inoculations.*

| Cold Virus Generation | Human Inoculation tests | Inoculated Groups | | | Control Groups | | |
|---|---|---|---|---|---|---|---|
| | | No. Subjects Used | No. Subjects Contracting Colds | % Contracting Colds | No. of Individuals | No. Exhibiting Colds | % Exhibiting Colds |
| 2 | 4/7/31 | 3 | 2 | 66 | | | |
| 4 | 4/23/31 | 5* | 3 | 60 | | | |
| 6 | 5/8/31 | 5 | 4 | 80 | 717 | 71 | 10 |
| 11 | 6/8/31 | 6 | 4 | 66 | 700 | 56 | 8 |
| 13 | 6/16/31 | 4 | 3 | 75 | 496 | 50 | 10 |
| 18 | 7/21/31 | 5 | 3 | 60 | 210 | 16 | 8 |
| 27 | 11/11/31 | 4* | 3 | 75 | 200 | 10 | 5 |
| Total | | 32 | 22 | 69% | 2323 | 203 | 8¾% |

* In each of these instances the person conducting the intranasal inoculation experiments was exposed to the cold virus being used, and in each case contracted a head cold at the same time as the positive test subjects. The number of subjects used in either case, however, does not include the operator.

oculation colds. Of the 22 individuals who developed colds as a result of inoculation 19 had moderately severe to severe head colds, running the usual course without complications. Two individuals had colds of comparatively short duration and 1 developed a mild bronchitis which was treated medically with uneventful recovery. While the incidence of colds in the inoculated cases was 69%, the incidence of colds in 2323 control individuals was less than 9%.

The length of time which elapsed between the preparation of the Seitz filtrate and its inoculation into test subjects was less than a day in the 6th, 13th, and 18th generations, was 2 days in the 4th generation, 3 in the 11th generation, 4 in the 2nd generation, and 8 in the 27th generation. It is particularly interesting to note that at the 27th generation, 7 months after the isolation of the cold virus strain, the incidence of infection was 75% and the resulting colds of more than usual severity. Since the 27th generation gave a high incidence of colds of unusual severity it is obvious that the virus has remained unimpaired in the Seitz filtrate at ice-box temperatures for a period of at least 8 days.

Since the dilution of the filtrate was 1 to 10 for each generation, the total dilution of the original virus culture at the 27th generation was 1 to $10^{27}$. The maintenance of this culture at full virulence for 27 generations and so long a period as 7 months fully confirms the conclusion reached by Dochez that the virus of the common cold may be readily cultivated in an appropriately prepared chick embryo medium and readily transmitted to suitable human test subjects.

Immune phenomena observed in the course of these experiments will be made the subject of a future communication.

5873

## Salt as a Factor in Edema Formation Following Plasmaphoresis.*

GEORGE FAHR, ARTHUR KERKHOF AND ELLIS GIERE.

*From the Department of Medicine, University of Minnesota, and the Minneapolis General Hospital.*

It is well known to clinicians that it is sometimes possible to rid a patient with nephrosis or with nephritis of visible edema by the deprivation of salt. When even moderate quantities of salt are ingested, visible edema may appear in 24 to 48 hours. There is a close correlation between edema formation and reduction of the colloid osmotic pressure in these cases consequent upon loss of protein from the blood plasma.

Leiter,[1] and Barker and Kirk[2] have bled dogs, thrown away the plasma and returned the cells suspended in Ringer's solution to the vascular system. Massive edema was obtained when the protein content of the blood was reduced. They obtained massive edema at protein values frequently higher than we have observed in our patients with edema. We believe this was because they gave large quantities of salt in physiological salt solution each day throughout their experiment. They gave 1500 cc. of physiological salt solution by mouth each day, equalling 13.5 gm. of NaCl per day, equivalent to about 60 gm. a day in an adult.

We have bled 7 dogs (500 cc. or more 3 times a day) removed the plasma which was replaced by Ringer's solution and returned the corpuscles to right heart. Only a very slight edema of the gluteal folds and the skin of the extremities was obtained when the total protein content of plasma was below 3% and osmotic pressure of the plasma colloids below 10 mm. Hg. The dogs were given 1500 cc. of tap water each day and the protein content held at close to the above figures. The dogs put out nearly as much water each day as was ingested. On giving 1500 cc. of 0.9% salt solution the output of urine immediately fell far below the intake and mas-

* Carried out with the aid of a grant from the Emma Sachs Plotz Fund.

1 Leiter, L., *Arch. Int. Med.*, 1931, **48**, 1.

2 Barker, M., and Kirk, E. J., *Arch. Int. Med.*, 1930, **45**, 319.