from the thyroidectomized animals of Baume *et al.*(4).

The increased sensitivity of the eruptive mechanism to thyroid hormone manifested by the PTU rats is probably due to the hypothyroid state of these animals and thus the end organs which are normally affected by this hormone become more reactive.

PTU causes a chemical thyroidectomy and produces a retardation of the I.E.R. which confirms the work of Baume *et al.*(4) in demonstrating a decreased eruption rate in the thyroidectomized adult rat. Since PTU does not affect the parathyroid gland, it is evident that the reduced I.E.R. can only be due to the lack of thyroid hormone.

*Conclusions.* Thyroid hormone is essential for a normal rate of incisor eruption. An excess of this hormone will cause an increased eruption rate. The use of the incisor eruption rate as a tool for demonstrating hormonal activity should be considered.

1. Hoskins, M., PROC. SOC. EXP. BIOL. AND MED., 1927, v25, 55.

2. Karnofsky, D., and Cronkite, E. P., *ibid.*, 1939, v40, 568.

3. Farris, E. J., and Griffith, J. Q., *The Rat in Laboratory Investigation*, Philadelphia, Lippincott, 1949, p152.

4. Baume, L. J., Becks, H., and Evans, H. M., *J. Dental Research*, 1954, v33, 80.

5. Schour, I., and van Dyke, H. B., *Am. J. Anat.*, 1932, v50, 397.

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## Relative Pathogenicity of Newly Isolated Strains of Poliomyelitis for Monkey Kidney Cells and Mice. (22126)

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Adaptation of poliomyelitis viruses to passage in mice has been accomplished only with difficulty or after laboratory manipulation of newly isolated strains(1-7). Quantitative comparisons of the pathogenicity of poliomyelitis viruses in tissue culture and in animals have usually been carried out with well adapted laboratory strains(8-11) or have been limited because of the necessary use of large numbers of monkeys(12).

The purpose of this study is to compare the quantitative relationships of the ability of freshly isolated polio virus strains to cause cytopathogenic effect in monkey kidney epithelium in tissue culture and paralysis in mice. For this purpose a number of strains of each of the three types of polio virus isolated in tissue culture from stools of patients during a national survey in 1952 was available for this study(13).

*Materials and methods.* Ten percent suspensions of stools were prepared in a mortar with phosphate buffered saline containing 2500 units of penicillin and 1250 μg of streptomycin per ml, clarified by centrifugation at 8,000 r.p.m. for one-half hour, and the supernates used as inocula. Primary isolations were made in cultures of monkey kidney, either in flasks of suspended tissue fragments, according to the method of Weller, Enders, Robbins and Stoddard(14), with subsequent passage in roller tubes, or directly in monocellular layer roller tubes, prepared by the method of Youngner(15). Isolates were typed in roller tubes by neutralization with specific monkey antisera. All inoculations of mice and titrations in both mice and kidney roller tubes were carried out on virus strains in the first or second tissue culture passage from the original stool. Groups of ten 4-5-week-old CFW mice were inoculated intraspinally (I.S.) with 0.02 ml, and observed for 21 days. Type 2 isolates were also inoculated intracerebrally (I.C.), in a dose of 0.03 ml, into 10 mice each, which were observed for 28 days. Wherever indi-

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TABLE I. Results of Mouse Inoculation of Polio Strains in 1st or 2nd Monkey Kidney T.C. Passage.

| Type | Intraspinal No. tested | Intraspinal No. pos. | Intraspinal % pos. | Intracerebral No. tested | Intracerebral No. pos. | Intracerebral % pos. |
|---|---|---|---|---|---|---|
| 1 | 51 | 0 | 0 | — | — | — |
| 2 | 56 | 45 | 80 | 56 | 11 | 20 |
| Original stools | 39 | 6 | 15 | 6* | 1 | 17 |
| 3 | 20 | 0 | 0 | — | — | — |

* All 6 were positive by I.S. inoculation.

cated, tissue culture harvests were titrated by the I.S. route, using 10 mice for each serial 10-fold dilution in saline. Fifty percent end-points were calculated by the method of Reed and Muench and the results expressed as titer per 1 ml of tissue culture supernatant. Parallel tissue culture titrations were performed in monocellular layer kidney roller tubes with Type 2 strains from the same tissue culture passage material used for inoculation of mice. Tenfold serial dilutions were made in Hanks' balanced salt solution (without bicarbonate), and 0.1 ml inoculated into each of 2 tubes, which were observed for cytopathogenic effect for 7 days. Results are expressed as titer per 1 ml of fluid.

*Results. Isolations in mice.* A. *Types 1 and 3.* Fifty-one Type 1 and 20 Type 3 isolates were inoculated into mice I.S. In view of the entirely negative results, as shown in Table I, I.C. inoculation of mice and titrations in tissue culture was not performed. B. *Type 2.* Harvests from the first or second passage in kidney tissue culture of Type 2 viruses from 56 patients produced paralysis in mice by the I.S. route in 45 instances (80%). (Table I) The average incubation period to paralysis of the first mouse in all groups receiving the undiluted tissue culture virus was 3.8 days.

These same 56 tissue culture passage fluids were also inoculated into mice by the I.C. route and only 11 (20%) were positive. Ten produced paralysis in only one or 2 mice out of 10, and one paralyzed all 10 mice inoculated. The average incubation period to first symptoms was 7.5 days (including one group in which the single positive mouse became paralyzed on the 27th day).

*Direct inoculation of stool suspensions into mice.* Thirty-nine stool suspensions were tested intraspinally. As shown in Table I, direct isolation of Type 2 strains was possible with 6 (15%), although no more than one or 2 animals of 10 inoculated developed paralysis. Virus concentration in the stool suspensions was not determined by titration in tissue culture. However, no correlation was apparent between ease of isolation directly from the stools and the titer of first passage tissue culture fluid either in mice or in tissue culture. All positive isolations directly from stool were from specimens which were also positive in mice after tissue culture passage. Six stools, positive by the I.S. route, were tested intracerebrally. One produced paralysis in a single mouse. The identity of this isolate was proved by specific neutralization with Type 2 serum.

*Relationship between I.S. titer and I.C. results in mice with Type 2 isolates.* Table II shows a comparison of the I.S. and I.C. routes of inoculation. No fluid was positive by the latter which had not produced paralysis by the former. A correlation between I.S. titer and I.C. pathogenicity is suggested by the fact that all 8 samples with I.S. titers greater than $10^{-4}$ were positive by the I.C. route, whereas only one of 18 with titers less than $10^{-2}$ was similarly positive. The one strain that paralyzed all mice inoculated I.C. titered $10^{-5.5}$ by the I.S. route.

*Relationship between titers in tissue culture and I.S. in mice.* In Table III the pathogenicity for monkey epithelium cells as indicated by titer in tissue culture and for the mouse spinal cord as evidenced by the I.S. titer is compared. There appears to be no significant correlation between the two since at any level of pathogenicity for one system there is a wide spread of the titer end-points

TABLE II. Relationship between I.S. Titer and I.C. Results with Type 2 Strains.

| I.S. titer | I.C. results Positive | I.C. results Negative |
|---|---|---|
| >4* | 8 | 0 |
| 3–4 | 1 | 9 |
| 2–3 | 1 | 8 |
| <2 | 1 | 17 |
| 0 | 0 | 11 |
| Total | 11 | 45 |

* Reciprocal of log titer/ml.

TABLE III. Comparison of Tissue Culture and Mouse I.S. Titers with Type 2 Strains.

| T.C. titer | I.S. titer Neg. | <2 (undil.) | 2–3 | 3–4 | >4 | Total |
|---|---|---|---|---|---|---|
| >7 | 3 | 2 | 1 | 0 | 4 | 10 |
| 6–7 | 5 | 7 | 4 | 7 | 3 | 26 |
| 5–6 | 2 | 7 | 2 | 3 | 1 | 15 |
| 4–5 | 1 | 2 | 2 | 0 | 0 | 5 |
| Total | 11 | 18 | 9 | 10 | 8 | 56 |

All titers expressed as the reciprocal of the log titer/ml.

by the other. Both first and second passage harvests from kidney roller tubes were tested in mice with ten strains. With 3, both were positive in high titer by the I.S. route. Of the remaining 7, 4 converted from negative to positive, but with low titers; 2 remained negative. Again, no correlation between tissue culture titer and the results in mice was demonstrable.

*Relationship of clinical type of disease in patient source of virus and pathogenicity of strains.* There were insufficient numbers of Type 2 virus strains from non-paralytic(11) and minor illness(8) patients to allow any significant comparison of titer results between these groups and the larger group of paralytic cases. However, while there appeared to be no difference in the tissue culture titers between these 3 groups, the strains from minor illness patients tended to have lower pathogenicity for mice than those from paralytic and non-paralytic cases.

*Discussion.* The entirely negative results obtained in the present study with 51 Type 1 and 20 Type 3 strains confirm the fact that the mouse pathogenicity of these immunologic types is extremely low.

Contrary to the difficulties encountered by earlier workers in adapting strains of Type 2 polio virus to mice by the I.C. route, the present study demonstrates the ease with which this can be accomplished with recently isolated strains when mice are inoculated I.S. Of a total of 56 strains tested by both routes, 20% were positive I.C., while 80% produced paralysis by the I.S. route. In a previous publication, one of the authors(16) showed a difference of approximately a log in titer between the two routes. In the present study all strains with I.S. titers greater than $10^{-4}$ per ml produced paralysis by the I.C. route, while only 8% of those with I.S. titers less than $10^{-4}$ were similarly positive.

That the high proportion of positive results reflects increased susceptibility of mice to I.S. inoculation, rather than increased concentrations of virus obtained in tissue culture, is brought out by the results of parallel titrations in the two systems. No correlation between tissue culture and mouse titer could be demonstrated. Four of five isolates in which the tissue culture titer was less than $10^{-5}$ produced paralysis in mice. On the other hand, of 36 fluids with tissue culture titers greater than $10^{-6}$, eight were entirely negative in mice.

It is of some interest to note that direct primary isolation was possible in mice with 6 of 39 stool suspensions tested I.S., and that in one case, primary isolation was accomplished by the I.C. route. Five were obtained from paralytic cases, and one (that positive I.C.) from a non-paralytic case.

Numbers of specimens available from different classes of clinical disease were too small for significant comparisons, but the Type 2 strains isolated from those with minor illness tended to be low in their pathogenicity for mice by the I.S. route. Further investigation of this relationship between severity of clinical disease and pathogenicity must await the availability of larger numbers of strains.

*Summary.* Polio virus in tissue culture fluids from first or second passage in monkey kidney roller tubes was tested in mice. Fifty-one Type 1 and 20 Type 3 strains were entirely negative when inoculated I.S. Forty-five of 56 Type 2 strains produced paralysis by the I.S. route, and 11 by the I.C. route. Those having the higher titers by I.S. route tended to be positive I.C. No correlation between tissue culture titer and mouse I.S. titer was apparent. Six strains of Type 2 were isolated directly in mice from 39 stools tested.

1. Armstrong, C., *Pub. Health Rep.,* 1939, v54, 2302.

2. Schlesinger, R. W., Morgan, I. M., and Olitsky, P. K., *Science,* 1943, v98, 452.

3. Melnick, J. L., and Ward, R., *Fed. Proc.,* 1948, v7, 308.

4. Koprowski, H., Norton, T. W., and McDermott,

W., *Pub. Health Rep.*, 1947, v62, 1467.
5. Bodian, D., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 1.
6. Li, C. P., and Habel, K., *ibid.*, 1951, v78, 233.
7. Li, C. P., and Schaeffer, M., *ibid.*, 1953, v82, 477.
8. Koprowski, H., Jervis, G. A., Norton, R. W., and Pfeister, K., *ibid.*, 1954, v86, 238.
9. Li, C. P., and Schaeffer, M., *ibid.*, 1954, v87, 148.
10. Krech, V., *ibid.*, 1954, v86, 488.
11. Wenner, H. A., *Ann. N. Y. Acad. Sci.*, 1955, v61, 840.
12. Sabin, A. B., *ibid.*, 1955, v61, 924.
13. Shelokov, A., Habel, K., and McKinstry, D. W., *ibid.*, 1955, v61, 998.
14. Weller, T. H., Enders, J. F., Robbins, F. C., and Stoddard, M. B., *J. Immunol.*, 1952, v69, 645.
15. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.
16. Habel, K., *ibid.*, 1951, v76, 357.

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## Occurrence of Pantothenyl Cysteine in Natural Materials.* (22127)

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Pantothenyl cysteine (PA-cysteine)† has been recognized as an early intermediate in the biosynthesis of coenzyme A (CoA) from pantothenic acid (PA). A liver enzyme can convert PA-cysteine to pantetheine (LBF) (1). PA-cysteine has been shown to be a precursor of LBF in *Acetobacter suboxydans* and it is strongly stimulatory to this organism (2,3). It is of interest to determine whether PA-cysteine occurs naturally. Because of the need for specificity and ability to determine materials in small quantities, a bioautographic method was developed to determine various conjugates occurring in natural materials. A strain of *A. suboxydans* 621 was used. This bacterium will grow on most known PA conjugates, and is generally more sensitive to them than to free PA(2,4).

*Methods.* A synthetic medium previously described(5) with 2% agar was used for the bioautograph. The sterilized medium was inoculated with a heavy culture of *A. suboxydans at* 40° and poured into a sterile container to a depth of 6-8 mm. This container consisted of a 19 x 50 cm glass plate, which rested within a stainless steel frame 15 mm high. After agar had solidified, a developed paper chromatogram of PA conjugates was sterilized by treatment with ether, and then spread on agar surface. After 10 minutes, the paper was peeled off, agar plate covered with a sterile 19x50 cm glass plate and incubated at 30° for 24 hours. The top plate was then removed and agar surface sprayed lightly with a mixture of PA (10 γ/ml) and yeast extract (100 mg/ml). The glass plate was restored and the whole assembly again incubated at 30° for 24 hours. Aseptic conditions were preserved throughout. The bioautograph was then observed against a background of diffuse light. The PA-yeast extract mixture was introduced because *A. suboxydans* otherwise grows very slowly under conditions employed. This mixture stimulated growth throughout the entire agar plate but it exerted its greatest effect in areas where growth had already been initiated, *i.e.*, where prior contact had been made with the PA conjugates on the developed chromatogram. *A beef liver* preparation was used as source of naturally occurring PA-cysteine. It was prepared as follows: 15 g of cold beef liver were ground in a Waring blendor with 50 ml

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† The following abbreviations will be used throughout this paper: pantothenic acid = PA; coenzyme A = CoA; pantetheine = *Lactobacillus bulgaricus* factor = LBF; pantetheine-γ-phosphate = LBF-γ-phosphate; pantothenyl cysteine = PA-cysteine.