

technically difficult and can be done with ordinary laboratory equipment. PR8 and Lee viruses were concentrated from allantoic fluid by this method, and human beings were inoculated with a formalinized concentrate. The mean antibody level of the subjects 2 weeks after vaccination was at least as high as that which followed the injection of similar amounts of active virus concentrated by centrifugation.

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Use of Biotin for Stimulating Growth of Nerve Tissue and Other Cells *in vitro*.

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A rapidly increasing number of data show that biotin is of widespread metabolic significance for a wide range of living things, and the fact that this vitamin markedly increases the growth rate of yeast cells and bacteria indicates that it might have similar effects on other plant or animal cells. Our present limited knowledge about many of the viruses, particularly those of the neurotropic group, is due in large part to the difficulties encountered in trying to grow them *in vitro*. In view of the important relationship between the metabolic state of cells and the ability of viruses to infect them,¹ experiments were devised to test the effects of biotin on various cell types, especially nerve tissue, in an effort to apply the data thus obtained for initiating or improving the growth of viruses and rickettsiae *in vitro*.

The method used for studying the effects of biotin on living cells was a slight modification of a technic previously described, using hanging drop cultures.² A piece of tissue was removed, preferably from the midline of an embryo, and divided into two equally-sized (and usually, bilateral) halves. One piece was explanted into a clot made up of plasma, embryonic extract, and biotin in Tyrode solution. A control culture was made of the other half in a clot consisting of the same proportions of plasma, extract, and plain Tyrode solution. Since the biotin (obtained from the S. M. A. Corporation) was furnished in alcoholic solution, it was necessary to add an

¹ Plotz, H., *C. R. Soc. Biol.*, 1937, **125**, 603, 719.

² Hamilton, H. L., *J. Exp. Zool.*, 1941, **88**, 275.

equivalent amount of alcohol to the Tyrode solution used in control cultures. In most of the concentrations ($1/6 \gamma$ to $5/6 \gamma$ of biotin per cc), the amount of alcohol was too small to affect the results appreciably. Explants consisted of brain, spinal ganglia, skin, mesenchyme, and somite material from 4- to 7-day chick embryos and from mouse embryos of various ages up to parturition. Chicken plasma and chick embryonic extract were used in all cultures. The pairs of treated and control cultures were incubated at 37.5°C for 4 to 6 days, and the comparative amounts of growth were measured.

In a total of 106 pairs of explants (both chick and mouse) 65% showed a stimulation of growth of cells in the presence of biotin. Within certain limits, the amount of stimulation was in proportion to the concentration used. For example, in one series of explants, using chick tissue, the average increase in outgrowth produced by biotin cultures over controls during a 4-day period was 358μ for a concentration of $1/6 \gamma$ per cc and 453μ for $1/3 \gamma$ per cc. The increase (442μ) obtained with $5/6 \gamma$ per cc was not in proportion to the concentration, possibly because of inhibition by the alcohol in the biotin solution, since the control cultures, containing the same amount of alcohol, did not show as good growth as ordinary control cultures. Among the concentrations used, the optimum amount of growth occurred with $1/3 \gamma$ of biotin per cc (final concentration in the clot). Increased outgrowth was observed in epithelial cells, fibroblasts, muscle cells, and nerves.

A detailed study was made of the effects of biotin on nerve tissue, since the amount of growth obtained heretofore has been of limited extent and duration (See Levi for a résumé of this problem).³ In addition to an increase in axon or fiber regeneration, which was often 3 or 4 times that obtained in control cultures at a concentration of $1/3 \gamma$ per cc, active proliferation of nerve cell bodies occurred in some cases, apparently depending on the region of the brain which was sampled. True mitotic division, with the formation of spindle and chromosomes, was not observed, but there was much amitotic activity, resulting in the formation of a narrow zone of outgrowth.

These effects of biotin are apparent only on embryonic nerve tissue, before the primordia are completely differentiated. For example, fiber growth was obtained from all parts of brains of mouse embryos, but only from the cerebral hemispheres of a newborn mouse, and none at all from any part of a juvenile mouse brain. A similar progressive fixation was observed when attempts were made to transfer and subculture nerve cells. Active proliferation of

³ Levi, G., *Zeit. f. d. Gesamte Anat. (Erg. Anat.)*, 1934, **31**, 125.

cell bodies was observed only in the original cultures. Fiber regeneration occurred in four successive subcultures, made over a period of 25 days, but the extent of the outgrowth diminished with each passage. The decreased size of the explant with each transfer may be a partial cause for this latter effect, since a portion of the tissue is invariably lost at each transfer.

It may appear somewhat surprising that biotin in such dilute concentrations should have a marked effect on the proliferation of embryonic cells, in view of the large amounts of biotin known to be present in eggs.⁴ Presumably, the chick embryos, from which the embryonic extract was prepared, should contain quantities of biotin, unless the vitamin is used up by the cells as fast as it is absorbed from the yolk. However, it is not unprecedented to find that very minute amounts of biotin can produce a considerable effect. For example, Du Vigneaud, *et al.*,⁵ find that, in rats, the addition of 2-4 γ per day of biotin to an ordinarily protective diet was sufficient to increase greatly the incidence of tumors produced by the carcinogenic compound, "butter yellow."

In view of the fact that biotin is actively synthesized by many species of bacteria and molds and is apparently important in their nutrition,⁶ it is hoped that the vitamin may prove valuable in producing higher titers of virus or larger yields of rickettsiae in culture. Experiments are in progress to test these possibilities.

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Protective Action of Desoxycorticosterone Acetate and Progesterone in Adrenalectomized Mice Exposed to Low Temperatures.

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The early work of Wyman and Tum Suden¹ with autoplasmic trans-

⁴ Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.

⁵ DuVigneaud, V., Spangler, J. M., Burk, D., Kensler, C. J., Sugiura, K., and Rhoads, C. P., *Science*, 1942, **95**, 174.

⁶ Landy, M., and Dicken, D. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **46**, 449.

* This work was started at the Roche-Organon Laboratories, Nutley, New Jersey.

¹ Wyman, L. C., and Tum Suden, C., *Am. J. Physiol.*, 1929, **84**, 362.