products of a neutral laevorotatory compound precipitable at least in part by phototungstic acid. This latter substance has been identified as *l*-γ-hydroxyproline. It was characterized by its specific laevorotation, absence of amino nitrogen, phenylisocyanate derivative M. P. 170° and copper salt. The free acid appears identical with the product obtained by the hydrolysis of proteins. The mode of linkage of the hydroxyproline with hydroxyglutamic acid is under investigation.

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Growth of Rickettsia of Typhus Fever (Mexican Type) in the Presence of Living Tissue.

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In spite of several attempts to cultivate the virus of typhus fever by the method of tissue culture, it seems that such cultures could not be carried through repeated successive generations. The methods employed have been practically the same; *viz.*, the cultivation of tissues (generally brain and spleen) from typhus infected guinea pigs in homologous plasma. Thus Kuczynski[1] found the virus of typhus fever virulent for 4-19 days, Krontowski and Hach[2] for 8 days, Wolbach and Schlesinger[3] for 20 days (28 days by transferring the same piece of tissue into fresh medium), and Rix[4] for 6 days. Recently Zinsser and Batchelder,[5] using tunica tissue from testicles of guinea pigs infected with Mexican typhus, prepared cultures which were virulent for one week. They were able to demonstrate rickettsias in great numbers in smears from such cultures.

The strain of typhus used in our studies, was isolated from a case in the southeastern United States by the Hygienic Laboratory in Washington, D. C. This strain is in all respects quite similar to the Mexican strain of Mooser. While not all of our attempts to

[1] Kuczynski, Max H., *Berl. klin. Wochenschr.*, 1921, **2**, 1489.

[2] Krontowski, A. A., and Hach, I. W., *Münch. med. Wochenschr.*, 1923, 144; *Klin. Wochenschr.*, 1924, **2**, 1625; *Arch. f. exp. Zellforsch.*, 1926-27, **3**, 297; *Z. f. Immunitätsforsch.*, 1927-28, **54**, 237.

[3] Wolbach, Burt and Schlesinger, Monroe, J., *J. Med. Res.*, 1923-24, **39**, 231.

[4] Rix, Erich, *Z. f. Hyg. and Infek.*, 1928, **108**, 103.

[5] Zinsser, Hans, and Batchelder, Albert P., *J. Exp. Med.*, 1930, **51**, 847.

establish strains by the methods to be described were successful, the following results were obtained in a number of experiments.

Employing the technic of Rivers, Haagen and Muckenfuss[6] for the cultivation of the viruses of vaccinia and herpes in tissue cultures of rabbit cornea in coagulated plasma, it was possible to carry the virus of typhus fever through at least 7 generations covering a period of 10 weeks. Pieces of normal guinea pig tunica (about 2 mm. square), soaked for a few minutes in a saline suspension of tunica scrapings from guinea pigs infected with typhus, were imbedded in wide tubes in a medium of guinea pig plasma coagulated by means of saline extracts of normal guinea pig spleen.

The successful cultivation of vaccinia virus by Maitland and Maitland[7] and by Rivers, Haagen and Muckenfuss[8] in a liquid medium composed of Tyrode solution, serum and minced tissue, suggested the possibility of using this same medium for the cultivation of typhus virus. Thus cultures were prepared as follows: minced normal guinea pig tunica, inoculated with typhus tunica scrapings as for the piece cultures above, was suspended in the serum-Tyrode medium of Maitland and distributed in about 3 cc. amounts in 25 cc. Erlenmeyer flasks (as suggested by Rivers). This medium was found to be as satisfactory for the growth of typhus virus as the coagulated plasma medium used above for the single piece cultures. By this method the virus was carried through 6 generations covering a period of 8 weeks.

Although our experiments were interrupted during the summer, it would seem that the cultures could be continued in either way indefinitely.

Transfers for both types of cultures were made at 8-12 day intervals by removing the tissue from the medium and inoculating fresh normal tunica with a small amount of the cloudy liquid obtained by scraping the issue of the previous culture.

Even the last generations of the cultures showed large numbers of rickettsias in Giemsa stained smears, the microscopical picture being like that published by Zinsser and Batchelder.[5]

Entirely characteristic infections with good scrotal swelling could be produced in guinea pigs upon intraperitoneal injection of single pieces of tissue (about 1 mm. square) from both types of cultures whenever tested; *e. g.*, from the fifth generation Maitland cultures.

[6] Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exp. Med.*, 1929, **50**, 665. *Cf.* Carrel, Alexis and Rivers, T. M., *Compt. rend. Soc. Biol.*, 1927, **96**, 848.

[7] Maitland, H. B., and Maitland, M. C., *Lancet*, 1928, **2**, 596.

[8] Rivers, Haagen and Muckenfuss, *J. Exp. Med.*, 1929, **50**, 181.

The organisms described by Mooser were found in large numbers in smears made from the tunica exudate of these animals.

The question of whether live tissue is necessary for the growth of typhus organisms, as it is supposed to be in the case of filterable viruses (*cf.* Rivers), is being studied by using tissues killed in various ways; *e. g.*, by heating, by repeated freezing and thawing, by anaerobiosis, etc.

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The Excretion of Xylose by Glomerular and Aglomerular Kidneys.

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Marshall[1] in a comparative study of the function of the glomerular and aglomerular kidney found that glycosuria is easily produced in fish with glomerular kidneys, but only a trace of glucose ever appears in the urine from an aglomerular kidney, even when the blood sugar is high and phlorhizin is given. This observation with those of Corley[2] and Fishberg[3] on the rate of disappearance of xylose from the blood suggested that a foreign sugar such as xylose might serve as a basis for measuring the extent of filtration and reabsorption by the kidney. First it was necessary to establish that xylose is not excreted by the aglomerular kidney except in the very faintest traces. That is the object of this paper.

Four species of fish were selected. The cod fish *(Gadus callarias)* and the puffer *(Spheroides maculatus)*, fish with glomerular kidneys; the toadfish *(Opsanus tau)* and the goosefish *(Lophius piscatorus)*, fish with aglomerular kidneys, were studied. The experiments on the goosefish were performed by Dr. E. K. Marshall, Jr., at Salisbury Cove, Maine. The other fish were obtained at the New York Aquarium. The urinary papilla was tied off and xylose in aqueous solution was injected into the posterior dorsal muscles in all experiments except those on the goosefish in which the xylose was injected intravenously.[4] At the conclusion of the experiment, 1 to

[1] Marshall, E. K., Jr., *Am. J. Phys.*, 1930, **84**, 1.

[2] Corley, R. C., *J. Biol. Chem.*, 1926, **70**, 521.

[3] Fishberg, E. H., *J. Biol. Chem.*, 1930, **86**, 665.

[4] Marshall, E. K., Jr., and Grafflin, A. L., *Bull. Johns Hopkins Hosp.*, 1928, **43**, 203.