acid has been demonstrated to serve as a growth factor for certain bacteria. It has previously been reported as an essential growth factor for *Corynebacterium diphtheriae* (Mueller and Klotz,[4] Evans, Handley and Happold[5]), for the lactic and propionic acid bacteria (Snell, Strong and Peterson[6]) and for the hemolytic streptococcus (Subbarow and Rane,[7] and McIlwain[8]).

Summary. Evidence has been presented which indicates that pantothenic acid and nicotinic acid are the essential growth factors for *Proteus morganii.*

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Urea-Treated Virus as a Vaccine against Rabies.*

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Urea and allied compounds have been studied with regard to their denaturing action on proteins[1, 2] and their inactivating effect on viruses.[3, 4] MacKay and Schroeder mixed rabies-fixed virus and poliomyelitis virus with concentrated urea solutions; complete inactivation of these viruses and loss of antigenicity resulted. The work here reported concerns the action of urea solutions on rabies-fixed virus.

In the first experiment fresh rabbit-fixed virus was ground with a saturated (room temperature) solution of urea in normal saline to make a 50% suspension of tissue. The resulting mixture was smooth

4 Mueller, J. H., and Klotz, A. W., *J. Am. Chem. Soc.*, 1938, **60**, 3086.

5 Evans, W. C., Handley, W. R. C., and Happold, F. C., *Brit. J. Exp. Path.*, 1939, **20**, 396.

6 Snell, E. E., Strong, F. M., and Peterson, W. H., *J. Am. Chem. Soc.*, 1938, **60**, 2825; *J. Bact.*, 1939, **38**, 293.

7 Subbarow, Y., and Rane, L., *J. Am. Chem. Soc.*, 1939, **61**, 1616.

8 McIlwain, H., *Brit. J. Exp. Path.*, 1939, **20**, 330.

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1 Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1929, **13**, 121.

2 Greenstein, J. P., *J. Biol. Chem.*, 1938, **125**, 501.

3 Stanley, W. M., and Lauffer, M. A., *Science*, 1939, **89**, 345.

4 MacKay, E. M., and Schroeder, C. R., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 74.

and creamy and flowed easily. Numerous bubbles of fat floated to the surface and were removed. The remaining material was homogeneous (save for small fragments of fibrous tissue, bits of blood clot and insoluble particles of a fatty consistency, all of which were removable by straining through gauze) and could be drawn through a 27 gauge needle. In 4 subsequent experiments less urea was utilized; these vaccines (Nos. 2, 3, 4 and 5) contained 40% rabbit-fixed virus brain and 60% of ⅓ saturated urea in normal saline. All vaccines, after preparation, were incubated at 37°C for 24 or 48 hours and were subsequently preserved at refrigerator temperature.

The undiluted vaccines of the first 2 experiments proved extremely irritating and rapidly killed a number of mice, when injected intracerebrally in doses of 0.02 cc to 0.03 cc in order to test for the presence of active virus. Three mice, which survived the initial cerebral irritation resulting from these test injections and which thereafter remained normal, indicated that the first vaccine was non-infectious for mice under the experimental conditions employed. The second vaccine killed all test mice shortly after intracerebral inoculation; its active virus content was, therefore, not determined. The last 3 vaccines were diluted 5 times with normal saline, before intracerebral inoculation, in order to minimize their direct irritating effects and injection of these vaccines produced no immediate untoward symptoms. The third and fifth vaccines contained active virus and produced rabies in a number of these test mice. Vaccine No. 4, however, was non-infective for 8 test animals, which were observed for a period of 18 days and which were subsequently shown to be fully susceptible to intracerebral infection with small doses of rabies virus. It was, therefore, concluded that vaccine No. 4 contained either no active virus or that its concentration of infective material was reduced to a minimum.

All 5 vaccines were tested for their immunizing efficacy. Vaccine No. 1 (which contained a high concentration of urea) was administered intraäbdominally in 8 daily doses, each of 0.025 cc, to 14 white Swiss mice. An intracerebral titration with fixed virus performed on these mice 2 weeks after active immunization was begun and compared with adequate untreated controls, indicated that no demonstrable immunity had developed. The last 4 vaccines, which contained less than half the amount of urea that was present in vaccine No. 1, showed very definite immunizing properties, however, when injected in like manner. Vaccines Nos. 3, 4 and 5 were injected in 2 different ways: (a) in 8 daily intraperitoneal doses of 0.025 cc, (b) in 1 dose of 0.20 cc. Controlled intracerebral titrations for immunity were

performed as above. The samples of rabbit brain-fixed virus, employed in these titrations, were removed from 50% glycerine in the first 4 experiments, while fresh virus was employed in test No. 5. Virus was ground to an initial 1/5 suspension in the first 3 tests, whereas a 1/10 suspension was prepared for the last 2 tests. These initial preparations were centrifuged at a moderate speed for a few minutes and the supernatants were further diluted by tens. Intracerebral test doses consisted of 0.02 cc to 0.03 cc of these dilutions. Normal saline was used as a diluent in the first 2 experiments and distilled water was employed after this. All mice were observed for a minimum of 14 days before being classified as "survivors".

Table I summarizes experiments on the immunizing powers of the last 4 vaccines, all of which tests yielded comparable results.

The table indicates that 8 intraäbdominal injections of urea-treated vaccines (Nos. 2, 3, 4, and 5) containing 40% rabbit-fixed virus brain and 60% ⅓ saturated urea in saline, produced a high degree of immunity against 1 and 10 minimal infective doses of rabies-fixed virus and some immunity against even greater doses. In addition, 3 of these vaccines (Nos. 3, 4 and 5) when given in like total amounts but in one large dose, produced an immunity of a somewhat lower grade.

Summary. Rabbit brain rabies-fixed virus exhibited considerable resistance to the action of urea. This virus maintained its antigenicity in 4 experiments and its infectivity in, at least, 2 tests in the presence of a concentration of urea which was sufficiently high to liquefy most of the virus-containing brain material.

TABLE I.
Resistance of Mice Immunized with Urea Vaccine.
All vaccinated mice received the same total amount of vaccine.

Intracerebral test virus M.I.D.'s	Vaccine given in: Eight injections		One injection		Untreated controls	
	Survivors	Deaths	Survivors	Deaths	Survivors	Deaths
10,000	2	3	0	4	0	4
1,000	0	4	0	4	0	4
100	14	6	5	7	0	10
10	15	1	6	6	0	24
1	18	0	11	5	1	24