chilled embryos of Amblystoma are to be used in experimental morphology, for retarding of the normal rate of growth by temperatures much below the optimum for the species must introduce variables of unknown value. At the same time this method may be used as a tool in experimental morphology in problems related to those attacked by Gilchrist,[1] and, correlated with cytological studies, it should yield significant results in regard to structure-function relations in development. It is known that localized acceleration of development within a relatively even gradient of growth in the central nervous system of Amblystoma accounts for the origin of its chief functional centers.[2] The development of the medullary plate also appears to take place according to the same principle within a wide field of potentially nervous ectoderm. The "organizer" in this case, and possibly in others, may be only an accelerator. The use of the method here proposed should throw new light on problems of this nature.

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Virucidal (Rabies and Poliomyelitis) Activity of Aqueous Urea Solutions.

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In an attempt to bring the suspended particles of an aqueous spinal cord suspension containing rabies or poliomyelitis virus into more intimate contact with inactivating agents for vaccine production trials a "solution" of the cord was made with the aid of urea. The effect of urea in strong concentration on these viruses proved interesting. As first recorded by Spiro[1] and in more detail by Ramsden[2] urea in aqueous solution has a remarkable ability to "dissolve" proteins. When spinal cord of the rabbit or monkey, moist with saline, is placed in a mortar and urea crystals added the cord on trituration quickly passes into an opaque syrupy "solution" containing innumerable lipid droplets in suspension. In this manner a 50%

1 Gilchrist, Francis J., *J. Exp. Zool.*, 1933, **66**, 15.

2 Coghill, G. E., *J. Comp. Neurol.*, 1926, **40**, 47; 1926, **42**, 1; 1928, **45**, 227; 1933, **57**, 327; 1936, **64**, 135.

1 Spiro, *Z. f. Physiol. Chem.*, 1900, **30**, 182.

2 Ramsden, W., *J. Physiol.*, 1902, **28**, xxiii.

cord "solution" containing from 30% to saturation with urea is easily obtained.

Rabies Virus. Typical results are presented in Table I. The fixed rabies virus usually killed within 6-11 days after intracerebral inoculation. The non-urea treated virus suspension was a 20% concentration of cord and brain in saline. The urea treated preparation contained 50% of cord-brain and about 40% of urea. Rabbits 3 and 4 were injected intracerebrally within an hour after the preparation of the cord "solution". They were given a second injection of 0.2 cc. each of a urea-treated preparation 10 days after the first ones with no ill effects. Rabbits 5 and 6 were given injections of 0.1 cc. each of the non-urea-treated preparation 3 weeks after the first injections. Both of them died within a week. Rabbit No. 7 was given a weekly "vaccination" by a 1.0 cc. subcutaneous injection of the 50% urea-treated rabies cord preparation for 6 weeks and then inoculated intracerebrally with the untreated suspension. Death followed shortly.

TABLE I.

No.	Species	Weight Kg.	Treatment	Max. Temp.	Paralysis days	Death days
			Rabies Virus.			
1	Rabbit	2.1	0.1 cc. Rabies virus suspen.		6	7
2	"	2.4	0.2 " " " "		8	10
3	"	2.3	0.1 " Urea treated Rabies virus		0	0
4	"	2.9	0.2 " " " " "		0	0
5	"	3.1	0.3 " " " " "		0	0
6	"	2.8	0.2 " " " " "		0	0
7	"	2.6	0.2 Rabies virus suspen. after "vaccination"		7	0
			Poliomyelitis Virus.			
	Monkey					
8	*Macacus rhesus*	3.2	0.1 cc. untreated virus suspen.	105.9	7	11
9	" "	2.9	0.2 " " " "	104.3	6	7
10	" "	3.0	1.0 " urea treated virus	101.7	0	0
11	" "	4.1	0.5 " " " "	100.5	0	0
12	" "	2.6	0.5 " " " "	102.1	0	0
13	" "	3.3	1.0 " " " "	100.8	0	0

Acute Anterior Poliomyelitis Virus. Cords from monkeys dying after inoculation with M. V. (monkey passage) virus[3] were used for preparing an untreated 10% suspension in buffered saline and a 40% "solution" in 40-50% aqueous urea. The results in Table I show that a strong urea solution inactivates poliomyelitis virus. Two weeks after the injection of monkey No. 10 the animal was chloroformed and a brain suspension of the injected area injected

[3] MacKay, E. M., and Schroeder, C. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 373

intracerebrally into another *Macacus rhesus* which did not develop the disease. Ten days after the first injection an accelerating dose[4,5] of 1 cc. of the urea-treated preparation was injected into the brains of Nos. 12 and 13 and still no symptoms of poliomyelitis developed.

A female monkey weighing 4.8 kg. and a male monkey weighing 3.9 kg. were bled from the heart and their serums titered with a 5% poliomyelitis monkey cord suspension. In a 1-5 dilution the serum of both monkeys had antiviral substances to the extent that monkeys receiving the usual dose of the mixture by subdural injection were paralyzed but did not die. With a 1-10 dilution there was no protection whatever and both monkeys died. The 2 normal monkeys were then injected subcutaneously once a month for 3 months with 3 cc. of the urea-treated virus suspension. A month after the last injection, intracerebral inoculation with the regular virus suspension (0.2 cc.) resulted in their deaths in 7 and 10 days respectively. Serum from blood drawn just before these inoculations was titered just as before the "vaccination". At 1-5 dilution one monkey was paralyzed and the other died, while at 1-10 dilution both test monkeys died. We conclude from this that the strong solution of urea not only attenuates or dilutes the poliomyelitis virus in the sense that it is non-infective but actually destroys it, for it no longer possesses immunizing power.[6]

Urea is such a relatively inactive substance and certainly not a protoplasmic poison such as are most virucidal agents that it is in a way surprising that rabies and poliomyelitis viruses are killed so easily by urea solutions. Strong urea solutions have a certain degree of bactericidal activity[7] but we have found that the death of bacteria usually requires considerably more time than that in which it kills viruses. The simple osmotic effect of the urea solutions can hardly be the cause of the virus death for glucose solutions of equivalent osmotic value will not inactivate rabies virus in any such period of time. It is true that neutral and inactive as it is, urea like alkalies denatures protein when dissolving it[8] and this reaction may be associated with the death of the virus. This denaturation occurs in a very few minutes. It might be noted that the toxic globulin of crotalus venom or the vegetable protein ricin does not lose its toxicity when denatured by a strong concentration of urea.

4 Flexner, S., *Science*, 1931, **74**, 520.

5 Flexner, S., *Science*, 1933, **77**, 413.

6 Flexner, S., *Science*, 1935, **82**, 420.

7 Foulger, J. H., and Foshay, L., *J. Lab. and Clin. Med.*, 1935, **20**, 1113.

8 Hopkins, F. G., *Nature*, 1930, **126**, 328.