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Effect of N-Dichloroacetyl-dl-Serine on Regeneration in the Forelimb of *Diemictylus viridescens.** (28870)

LUCY GOMES (Introduced by Alexander Wolsky)

Division of Clinical Investigation, Sloan-Kettering Institute for Cancer Research, New York City

The urodeles among amphibians possess a high degree of regenerative capacity in adult life. Since the process or regeneration presents many of the phenomena of embryonic development, adult amphibians can be used for the study of developmental processes. It therefore seemed of interest to use regeneration blastemas as testing material for the study of the biological effect of the Na salt of N-dichloroacetyl-dl-serine, which was shown by Levi *et al*(1) to be carcinostatic in certain cases.

Material and methods. Adult salamanders of the species *Diemictylus viridescens* were obtained from commercial dealers in New York City and kept in finger bowls. They were fed tubifex worms for several days before the experiment.

Amputation of the forelimb was carried out at the distal end of the humerus. No narcotics were used for the amputation. The animals were placed in batches of 6 each in a finger bowl. An hour after amputation each of the animals was injected with 1 ml of the Na salt of N-dichloro-acetyl-dl-serine, made up in amphibian Ringer's solution. Two concentrations were used, 0.1 and 0.2 %, in 2 series of experiments. The injections were repeated daily throughout the experiment. A control series using 1 ml of amphibian Ringer's solution per day was also set up. In other controls no injections were used. Injections were given subcutaneously as near to the region of amputation as possible. When this was not possible (usually after about 15 to 20 days) intramuscular or intraperitoneal injections were given at any region of the body.

The animals were occasionally fed with tubifex worms during regeneration, but they did not eat regularly. The water in the bowls in which the animals were kept was changed every other day.

Camera lucida drawings were made of the regenerates at 35, 45, and 56 days after amputation. The experiment was then terminated.

The various phases of regeneration may be split into 4 distinct phases following the account of Hay and Fischman(2); (1) a wound-healing phase: within 24 hours after amputation, when the transected surface of the limb was covered with a well-defined wound epithelium; (2) a dedifferentiation phase: usually by the fifth day after amputation, when wound healing was complete and a disintegration of the damaged muscles was apparent; (3) a growth phase: following the establishment of a definitive blastema, when extensive dedifferentiation ceased and growth and mitosis became the dominant features of the regenerative process; and (4) a differen-

* The data in this paper are taken in part from a thesis, presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Fordham University.

tiation phase, in which cartilage was the first tissue to differentiate within the regeneration blastema, followed by early muscle anlagen as discrete bundles of elongate cells. The studies on the effect of the substance to be tested were made in comparison to this normal process.

Inhibition pattern. During the first phase of regeneration experimental animals did not differ from controls. By the 26th day, growth of the regenerates in the treated animals was slightly retarded. At this point, differences in the regenerates of the animals treated with the 2 different concentrations were also noted. The 0.2% solution had a stronger effect than the 0.1% solution.

By the 35th day of regeneration, differentiation of the digits began to appear in control animals, but in only 50% of the experimental animals of the 0.1% series. The 0.2% series did not show signs of digit differentiation.

On the 46th day of regeneration all 4 digits were formed in the control animals, and average length of the regenerates was 2.5 mm. Digit differentiation was still lagging behind the controls in animals treated with the serine analogue. The average length of the regenerates of the salamanders treated with 0.1% solution was the same as that of the controls, *i.e.*, 2.5 mm, and in a few cases they grew even better than the best controls. Animals injected with 0.2% N-dichloroacetyl-dl-serine showed definite retardation in growth. In the animals that survived to this stage average length of the regenerate was 2 mm, with little variation.

On the 56th day of regeneration all of the digits in all of the animals, both test and control, were differentiated. This period of regeneration is important because by this time the digits were fully developed in the controls and the regeneration process could be considered completed.

The average length of the regenerates in the control salamanders at this stage measured 2.9 mm. The average length of the regenerates of animals injected with 0.1% N-dichloroacetyl-dl-serine was almost the same, and again, in certain cases, the length exceeded the best control figures. The salamanders treated with a 0.2% solution showed definite retardation and inhibition in growth of the regenerates. Their average length, 2.35 mm, was considerably less than in the controls, although all 4 fingers of the limb were formed.

TABLE I. Growth of Regenerates 56 Days After Amputation.

Specimen	Control (amphibian Ringer's solution)	Treated (.2% N-dichloroacetyl-dl-serine)
	(Length of regenerates in mm)	
1	2.90	2.25
2	3.20	3.10
3	4.00	2.50
4	2.50	2.30
5	3.00	1.75
6	3.20	2.20
7	2.40	
8	2.60	
9	3.00	
10	2.40	
Mean	2.92	2.35
S.E. of mean	.158	.146
S.E. of diff.		.215
"t" value at .05 level = 2.65		

Table I gives details of the results with 0.2% solution on the 56th day, when the experiment was terminated. Statistical evaluation shows that the "t" value at the 0.05 level was 2.65 and the standard error of the difference 0.215, indicating that the antimetabolite at this concentration produced a statistically significant growth-inhibiting effect.

It might be concluded from these experiments that the 0.1% solution had no measurable effect on regeneration, except perhaps producing a slight delay in the appearance of digits. But the 0.2% solution of N-dichloroacetyl-dl-serine caused definite inhibition in regenerating limbs of salamanders, although no morphological abnormalities were observed. This inhibition can be attributed to the substance, since the extent of inhibition was correlated with the concentration used.

Discussion. The effects of N-dichloroacetyl-dl-serine used in these experiments were similar to those reported by others, who used ionizing radiations(3,4) on regenerating limbs. The substance is an amino acid analogue, and it is therefore likely that it in-

hibits growth by interfering with protein synthesis. This interference was seen morphologically when the blastema was formed. It may be that the analogue prevented growth of already formed cells, or it may have acted by cutting down the number of blastema cells. Externally the only sign of this inhibition of protein snythesis was retardation of growth, and the exact mechanism remains unknown.

No specific morphological abnormalities were encountered in the animals treated with N-dichloroacetyl-dl-serine. The 2 early stages of the regeneration process (*i.e.* the wound-healing and dedifferentiation phases) were not affected. But by the 25th day the growth of the regenerates in the analogue-treated animals was lagging behind that of the controls. Later there was also a slight delay in digit differentiation. Thus it seems that the process of regeneration was affected in the later stages, when the blastema had formed and growth and differentiation had begun.

Summary. The effect of N-dichloracetyl-dl-serine, when injected into regenerating forelimbs of salamanders, was observed. Regeneration was studied over a period of 56 days. Except for a decrease in growth of the regenerates no significant morphological abnormality was produced. A 0.2% solution of the substance proved more effective than a 0.1% concentration. The substance affected the regenerating tissues at the later phases of regeneration, namely active growth and differentiation.

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Interaction of Mycoplasma (PPLO) and Murine Lymphoma Cell Cultures: Prevention of Cell Lysis by Arginine.* (28871)

PAUL M. KRAEMER (Introduced by H. Koprowski)
Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

Preliminary characterization of several strains of mycoplasma that are cytocidal for L5178Y and P388 murine lymphoma cell lines has been presented(1). These strains were isolated from various types of cell cultures, but produced no discernible cytopathology in cell cultures other than the murine lymphoma cell lines. Many other mycoplasma strains such as T-5, Eaton agent, and other strains isolated from cell cultures were incapable of adversely affecting L5178Y cells carried *in vitro*.

In the earlier report, there were 3 indications that the mechanism of lysis of the L5178Y cells might not depend upon infection of the cells: first, yield of mycoplasma following inoculation of various types of cell cultures was not related to occurrence of cytopathology; second, time of lysis of a L5178Y cell culture was correlated with growth curve of the mycoplasma rather than with multiplicity of infection; finally, L5178Y cells grown in the ascites form in genetically susceptible mice were completely resistant to the lytic mycoplasma, even when the mycoplasma and cells were mixed at high multiplicity and preincubated prior to intraperitoneal injection.

The present paper reports results which indicate that the cytopathic effect produced by these lytic strains is independent of cellular infection and is, in fact, related to production of mycoplasmal "toxins" that deplete the medium of arginine.

Materials and methods. The basic techniques used for handling mycoplasma and L5178Y cells have been described(1). Briefly, the L5178Y cells are carried *in vitro* in "L-1"

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