Serine Derivative with Antitumor Activity

Abstract. The sodium salt of N-dichloroacetyl-DL-serine depresses the growth of sarcoma-37 in mice, causing complete regressions of the tumor. The compound is nontoxic to mice in doses up to 4 gm/kg of body weight. The animals do not lose body weight and show no alteration in the formed elements of the blood or in hemoglobin content.

Amino acid derivatives which might serve as antagonists in the metabolism of tumor cells have been investigated for many years (*1*). Since some antibiotics have the ability to destroy neoplastic cells in experimental animals (*2*), it was reasoned that a natural amino acid modified to contain a portion of a natural antibiotic may have antimetabolite properties. As a starting point we chose the N-dichloracetyl group of chloramphenicol, since the natural amino acids are easily acylated. We have synthesized a number of N-dichloroacetyl derivatives of α-amino acids and are investigating them for antitumor activity in experimental animals.

We wish to give at this time a brief account of preliminary experiments concerning the effect of one of these derivatives—namely, the sodium salt of N-dichloroacetyl serine—on tumor growth.

Commercial DL-serine was treated with dichloroacetyl chloride in an aqueous alkaline medium at 0°C, according to the usual procedure employed for N-acylating amino acids. The purified, colorless, crystalline N-dichloroacetyl-DL-serine melted at 119° to 121°C. The sodium salt of N-dichloroacetyl-DL-serine [Frosst-T-9045 (FT-9045)] was also a white crystalline compound and melted at 178° to 179°C (with slight decomposition). Both compounds were readily soluble in water at room temperature. The sodium salt was used in the experiments described below.

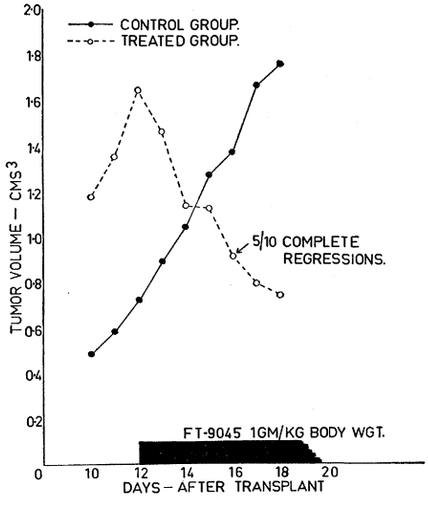

Fig. 1. Effect of 1 gm of the sodium salt of N-dichloroacetyl-DL-serine per kilogram of body weight per day on the growth of sarcoma-37 in mice. Each point represents the average tumor volume for ten animals.

Sprague-Dawley rats and Connaught mice tolerated doses up to 4 gm of the salt per kilogram of body weight, injected intraperitoneally, with no visible abnormal effects. None of the animals died. Connaught mice receiving a daily dose of 1 gm of the salt per kilogram of body weight, injected intraperitoneally for 7 days, showed no alteration in white blood cells, red blood cells, or hemoglobin content.

An anesthetized cat was given a continuous intravenous infusion of 1 ml of a 10-percent solution of the compound per minute. Blood pressure, respiration, and heart rate were recorded. At the end of 4 hours, 21 gm (7 gm per kilogram of body weight) had been infused. During this time there was no change in blood pressure, respiration, or heart rate. The cat responded physiologically to acetylcholine and to adrenaline at the end of the infusion. Autopsy revealed no gross abnormality.

Twenty Connaught mice with actively growing transplanted sarcoma-37 were divided into two groups of ten each. One group was kept as an untreated control. On the 12th day after subcutaneous transplant, the other group was injected intraperitoneally daily with 1 gm of the compound per kilogram of body weight. The volume of the tumors was determined daily by measuring three dimensions with calipers. The results are shown in Fig. 1. On the 6th day of treatment, five out of ten of the treated animals showed complete regressions of their tumors, and on the 25th day, seven out of the ten tumors had completely regressed. In the remaining three animals the tumors continue to regress. The tumors in the untreated control animals progressed in size, as was expected. The tumors in the treated animals became very small and hard during their regression and eventually sloughed off, leaving an ulcer which finally healed. Histological study of tumors on the 6th day of treatment in a parallel experiment showed extensive necrosis with a few isolated islands of tumor cells. Sections from untreated animals studied at the same time showed actively growing tumor cells with numerous mitoses. The lack of toxicity of this compound which has been shown in normal animals was again evident in the tumor-bearing animals. There was no weight loss such as one usually finds with toxic drugs (mustards and so on), and there were no deaths.

In view of the low toxicity of the sodium salt of N-dichloroacetyl-DL-serine and its apparent specific effect on neoplastic growth, this compound is now being investigated clinically on patients with advanced malignant disease.

IRVING LEVI
HAROLD BLONDAL
EZRA LOZINSKI
Research Laboratories, Charles E. Frosst and Company, Montreal, Canada

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Selective Uptake of Serum Globulins and Glycoproteins by Cells Growing in vitro

Abstract. It has been demonstrated in preliminary experiments that two strains of rat tumor cells (WRC-256 and TSAT-72) utilize alpha-2-globulin more selectively than other protein components of human serum. Cells of strain TSAT-72 show some utilization of beta-globulins also. Separate experiments demonstrated the utilization of glycoproteins by both strains.

Pathological manifestations in man and other mammals are very often reflected in the biochemistry of the circulating blood. Especially during the last decade, valuable information has been obtained by applying new electrophoretic methods to the study of the blood and serum components of normal and diseased subjects. Among the major constituents of the serum, large molecules as well as their conjugated forms such as lipo- and glycoproteins have been shown to fluctuate considerably in various physiological and pathological conditions.

The role of serum proteins in growth processes and particularly in neoplastic growth represents still another problem which needs to be investigated further. Tissue culture has provided a good tool for the study of the biological activity and the nutritional value of these complex macromolecules during the growth of mammalian cells in vitro. Jaquez and Barry (*1*) observed that the growth-promoting activity of serum is associated with such nondialyzable protein fractions as the globulins. We have already reported (*2*) the utilization of serum alpha- and beta-globulins by several malignant cell strains and a normal strain growing in tissue culture. Madden and Whipple's experiments (*3*) on the participation of serum proteins in the metabolism of cells show another example of the

utilization of these components by cells in vivo.

In recent experiments the rat tumor cell strains TSAT-72 sarcoma and Walker 256 carcinoma were grown in a culture medium consisting of 50 percent human adult or human placental cord serum and 50 percent Gey's balanced salt solution (*4*). The cultures were incubated at 37°C for 29 days without renewal of the medium in specially designed double diffusion chambers (*5*). The culture fluids were studied after periods of 6, 12, 20, and 29 days of continuous culture and growth.

Paper electrophoresis of the proteins and glycoproteins was carried out according to a procedure already described (*2*). In the experiments on strain WRC-256 it was found that alpha-2-globulins were utilized more rapidly than the beta fractions. The drop of the levels of alpha-2-globulin started around the sixth day. In more advanced stages of the cultures (26 days) without renewal of the medium, the levels of alpha-2-globulin were considerably lower than those of the beta fractions. By comparison, the rat sarcoma strain TSAT-72 utilized alpha- and beta-globulins equally under these experimental conditions. However, in the case of the Walker carcinoma 256, the uptake of alpha-2-globulin is more uniform and predominates over the beta uptake, which appears to be very slight. With WRC-256, even after 29 days of continuous culture in the same medium, the levels of beta-globulin remain almost identical to the levels found in the original unincubated fluid and the incubated fluid controls (no tissue blank). In these experiments a drop of 35 to 40 percent in the alpha-2-globulin was repeatedly observed (Fig. 1).

Since the group of alpha- and beta-globulins comprises several complex proteins such as lipo- and glycoproteins, the possibility that the protein-bound carbohydrates could be selectively utilized was investigated. It is known that serum alpha-2-globulin is very rich in carbohydrates. The strains TSAT-72 and 256 were used in these studies. Cultures were set up and maintained for as long as 29 days, as described. Paper electrophoresis of the bound carbohydrates and quantitative analysis by Winzler's method (*6*) of the culture fluids showed a considerable depletion of the glycoproteins at the end of 29 days (Fig. 2). The controls (no tissue blank) did not show any drop in the glycoproteins. The results with both TSAT-72 and WRC-256 are similar. Since the cells of both epithelial (WRC-256) and mesenchymal origin (TSAT-72) utilized significant amounts (approximately 35 percent) of the

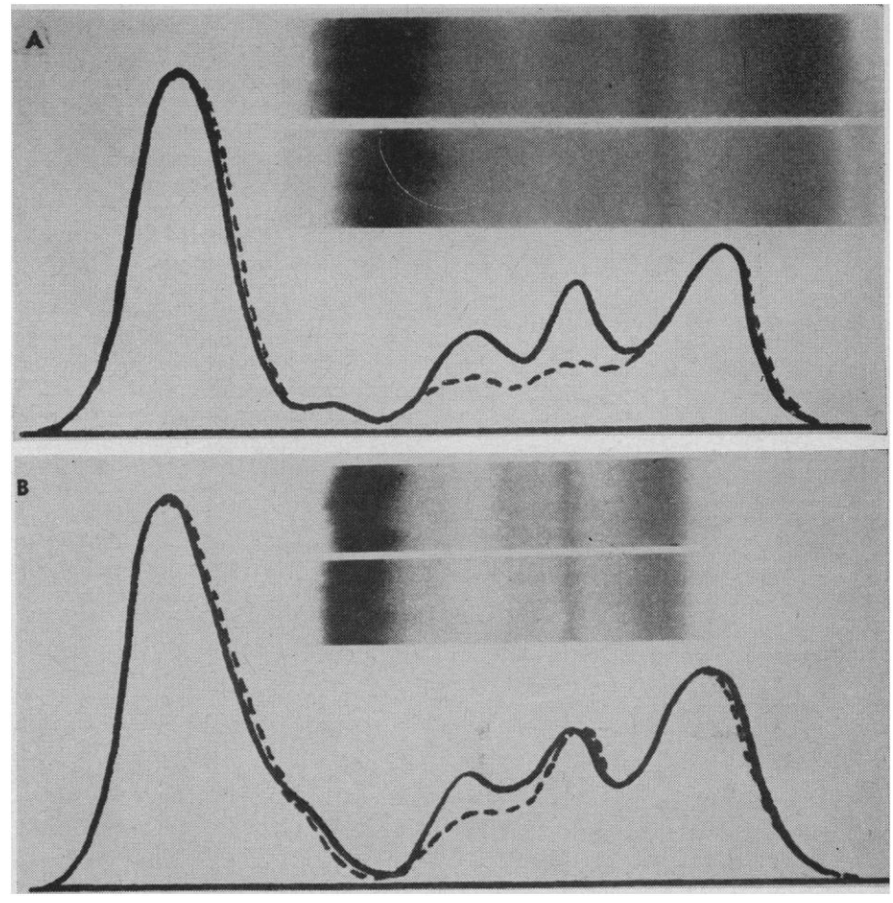

Fig. 1. Paper electrophoresis patterns and recordings of the serum proteins. Incubated fluid controls—no tissue blank (top patterns and solid curves) and culture fluids after 20 days (bottom patterns and broken curves). *A*, TSAT-72 rat sarcoma; *B*, WRC-256 rat carcinoma.

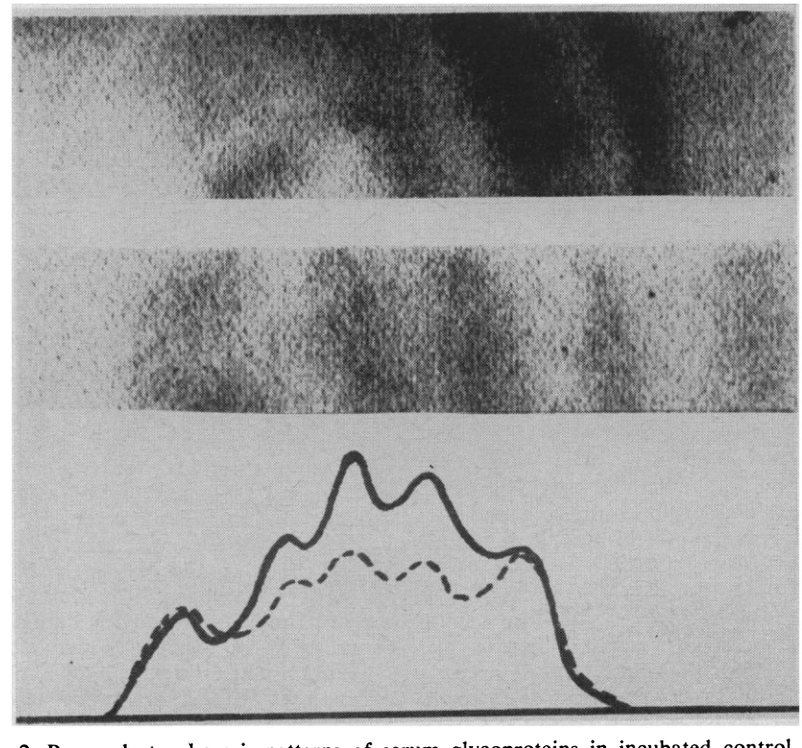

Fig. 2. Paper electrophoresis patterns of serum glycoproteins in incubated control fluid (top pattern, solid curve) and WRC-256 culture fluid (bottom pattern, broken curve).

available glycoproteins, it may be suggested that the alpha-2-globulins are important in the nutrition of mammalian cells.

H. NAIM KENT
GEORGE O. GEY

Department of Surgery, Johns Hopkins University Medical School, and *Department of Pathobiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland*

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4. This work was supported in part by a grant (P-23) from the American Cancer Society and in part by a grant (C-356) from the National Cancer Institute, U.S. Public Health Service.
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Distribution of Labeled Carbon in Reef-Building Corals with and without Zooxanthellae

Abstract. The uptake and distribution of carbon-14 in two reef-building corals, *Manicina areolata* and *Montastrea annularis*, were studied by radioautographic methods. Experiments on colonies with and without zooxanthellae were run in light and in darkness. We suggest that corals cannot derive any effective nutrition from their zooxanthellae, and that the physiological response of corals to the presence of zooxanthellae is due to the secretion of trace amounts of vitamin-like or hormone-like substances by the algal symbionts, and not to their food value per se.

Using a radioautographic method, Muscatine and Hand (*1*) reported transfer of photosynthetically fixed material from the zooxanthellae into the host tissues of the sea anemone *Anthopleura elegantissima* (Brandt). Although these investigators obtained no data about the nature and quantity of the material transferred, they suggested that the actiniarian host could derive at least a part of its nutrition from its commensal algae. Muscatine and Hand also claimed that their conclusions support the inference that the hermatypic, or reef-building, corals, which also contain zooxanthellae, could likewise derive food from the algae.

The point of view that hermatypic corals are herbivorous as well as carnivorous is a controversial one which has recently been resurrected by Sargent and Austin (*2*) and Odum and Odum (*3*) to explain results of their studies on the oxygen balance of Pacific atoll reefs. On the other hand, the experimental evidence of Yonge *et al.* (*4*, *5*) demonstrated that corals appear to be specialized carnivores which cannot utilize their zooxanthellae for food. These investigators also showed that the zooxanthellae are nevertheless very important in the bioeconomy of their hosts in that the metabolic efficiency of the coelenterate is greatly enhanced through the *in situ* absorption of waste products and the liberation of oxygen. Our own unpublished observations confirm these conclusions.

It is now almost certain that, among the Coelenterata, zooxanthellae do play an important role in the nutrition of some of the Alcyonaceae, notably the Xeniidae (*6*), and possibly in some of the Zoanthidea as well. There appears to be great variation throughout the various groups of the coelenterates, some of which are purely carnivorous even though they contain zooxanthellae, whereas others are wholly or in part herbivorous. The role played by the zooxanthellae in the bioeconomy of their coelenterate hosts appears to vary in specific and unpredictable ways from one group to another, and it is unjustified to extrapolate results from the Actiniaria to the Scleractinia, as was done by Muscatine and Hand.

Investigations are now in progress in this laboratory to determine the role of the zooxanthellae in calcium deposition and nutrition in the hermatypic scleractinia, or reef-building corals (*7*, *8*). In view of the possible importance of our findings in helping to resolve the controversy over zooxanthellae in reef corals, we present here some preliminary results on the fixation and migration of labeled carbon in corals with and without zooxanthellae, both in light and darkness.

Two species of West Indian reef-building scleractinian corals were used in our experiments, *Manicina areolata* (Linnaeus) and *Montastrea annularis* (Ellis and Solander). The latter is one of the most important framework builders of Western Atlantic reefs (*9*). Half of the colonies used were first kept in complete darkness for 3 months to cause complete extrusion of the zooxanthellae from the coenosarc; the other colonies used were normal and contained large numbers of zooxanthellae. One series of colonies, both with and without zooxanthellae, was run in light, 1 foot under a double bank of 40-watt daylight fluorescent tubes. Another series, containing the same types of coral, was run simultaneously under identical conditions, except that the colonies were kept in lightproof jars. $Na_2C^{14}O_3$ at *p*H 9.0 was added to the experimental vessels to give a final activity of approximately 1 μc/ml. The water was stirred with a gentle stream of moist air to prevent anaerobiosis and *p*H changes, especially in the dark jars. The experiments were run for 50 hours at a temperature of 27 $\pm$ 1.5°C and were terminated by transferring the corals into "cold" fresh-running sea water for 2 hours to rinse out any activity not incorporated into tissue constituents. The specimens were fixed in modified Carnoy's fluid (1 part glacial acetic acid to 3 parts absolute ethyl alcohol), decalcified *in vacuo* with 10 percent glacial acetic acid, washed, dehydrated, embedded, and sectioned at 5 and 9μ. Radioautography was carried out by the stripping film method of Pelc (*10*) with Kodak Ltd. (England) A.R. 10 plates. The emulsion was exposed for 10 days at 4°C and developed.

Intense exposure was observed over the zooxanthellae of those corals which were kept in the light, whereas there was virtually none in those which had been kept in darkness to prevent photosynthesis. The amount of activity incorporated was three to five times greater in zooxanthellae located in the gastroderm of the oral disk and column wall than in those located in deeper tissues such as the mesenteries and calicoblast, where the available light is of much lower intensity and the rate of photosynthesis is thereby reduced. Very little activity was seen in zooxanthellae located in the excretory zone of the mesenteries, possibly because these algae were either moribund or dead and in course of extrusion from the coral (*5*).

No significant algal uptake of radiocarbon was observed in those corals which had been kept in darkness. The tissues of both normal and zooxanthellaless corals kept in darkness during the experiment showed identical but faint background activity, probably due to the heterotrophic CO_2 fixation in the animal tissues. The tissue background activity of the zooxanthella-less corals kept in the light was equally low. In normal corals kept in light to allow algal photosynthesis, preliminary grain counts indicated that the tissue background activity was about five times higher than in the controls kept in darkness. This activity was confined to the cell masses of the epidermis and gastrodermis, and was reduced or absent over mucous glands and the mesoglea, which is nearly acellular. The activity was somewhat greater in tissues that contained zooxanthellae than in those that did not, but the difference was small. These observations indicate that some of the radioactivity fixed by algal photosynthesis was transferred and became incorporated in the tissues of the animal host.

The level of such transfer seems