Technical Papers

Cyanide Protection against X-Irradiation

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The recent report of Patt *et al.* in *Science* (*15*), showing a cysteine protection against x-irradiation, increases the interest of our observations on a similar protection by cyanide, reported March 26, 1949 at the Société Belge de Biologie (*5* and *6*).

Recent papers by French and English physical chemists showing the presence of hydrogen peroxide in irradiated water suggested experiments on frog's isolated *rectus abdominis* (*7*, *14*). This muscle, irradiated with therapeutic doses of x-rays or dipped in a solution containing P_{15}^{32} in form of phosphate, showed when stimulated with KCl a contracture very similar to that observed by Bacq (*1*, *2*) after the action of H_2O_2.

Dustin and Gompel (*10*) injected hydrogen peroxide intraperitoneally in mice and showed that it was a radiomimetic poison. These observations were in agreement with our general ideas on the actions of oxidizing agents as "thioloprive" substances (*3*, *4*). Frederic (*12*) has shown that the —SH groups disappear in the skin of the guinea pig after x-ray irradiation.

Two of us (*13*) succeeded with the semicarbazone of adrenochrome (Adrénoxyl Labaz), which increases capillary resistance in inhibiting the x-ray purpura in mice; but this substance does not change the mortality. Field and Rekers (*11*) with flavonids possessing vitamin P action, succeeded in decreasing x-ray mortality.

Striking results have been obtained with sodium (or potassium) cyanide. Our technique was as follows. Mice of pure breed (C 57 black or A.K.A.), weighing about 30 g and 4 to 6 months old, were irradiated by groups of 10, using 230 kv, 12 ma, copper filter 0.25 mm, focal distance 50 cm, field 100 cm², mean output 30 r per min. The 42 control mice receiving 500 r to 600 r all died between the 4th and the 8th or 9th day. Animals receiving 0.1 mg NaCN just before similar irradiation showed 50% to 80% survival. The same dose of NaCN given immediately after irradiation only delayed mortality. When the cyanide was injected 15 min after irradiation, the mortality curve was exactly the same as that of the controls (Fig. 1). A statistical analysis does not seem necessary to show that these results are highly significant. Our conclusions are based on the observation of 11 groups of 10 mice each. Cyanide is rapidly detoxicated in —SCN by an enzymatic system localized in the liver, but NaSCN either has no effect or shortens the survival period of irradiated mice. Thus it is the CN^- anion that is responsible for this protective action. We have not yet collected the various facts that would allow us to give a reasonable interpretation of this action of cyanide, but several possibilities may be discussed in the light of the similar

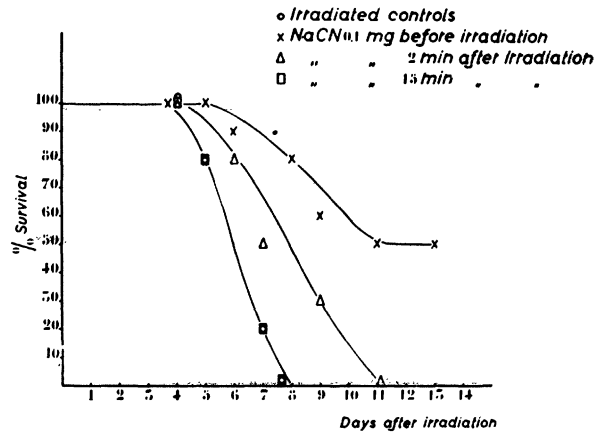

FIG. 1.

successful experience of Patt *et al.* with cysteine.

Cyanide reduces disulfide bonds (—S—S—) to sulfhydryl groups —SH. We do not believe, however, that the dose of cyanide injected is sufficient to give an effective reducing concentration; maximal tolerated amount of BAL (1.7 mg) injected in mice before irradiation only delays mortality; there is no permanent survival. One may suspect that the interpretation of the results of Patt *et al.* is not as simple as it seems at first sight, because preliminary experiments have shown in our laboratory that the level of reduced glutathione in blood and tissues (liver, kidney, heart, and muscle) of mice is not lowered after lethal irradiation. The failure of cyanide or cysteine to act when given after irradiation shows that the biochemical lesion *in vivo* is not as easily reversible as —SH enzyme inactivation *in vitro* (*8*, *9*).

Cyanide might simply reduce temporarily the metabolic activity of the animal. It is known that there is a certain parallelism between total metabolism and radiosensitivity: an increase of metabolism is associated with increased sensitivity, whereas relative resistance is observed with anoxic tissues.

Cyanide might also inhibit some heavy metal enzyme responsible for the rapid disposal of H_2O_2, thus decreasing the rate of reaction of this peroxide with some reducing substance in the tissues. Experiments are in progress to test these various possible interpretations.

Thus, we may conclude that cyanide, but not thiocyanate, protects a significant percentage of mice irradiated by a lethal dose of x-rays, that this poison is ineffective when given after irradiation, and that many more experiments are needed in order to be able to give a correct interpretation of these facts.

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Serological Relationships between Nucleus, Cytoplasm, and Cytoplasmic Products and the Concept of Complementary Molecules

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The concept of antibodies as units complementary to their antigens was originated by Breinl and Haurowitz (*2*). Pauling (*10*) has developed the idea of antibody specificity as resulting from the folding of initially stretched-out polypeptide chains over limited regions of the antigen. The molecules thus folded into specific configurations become stabilized by bonds acting between their folds, and thereafter "fit" the specific regions of the antigen. Such complementary molecules (antibodies) can subsequently combine with their antigens, since the configurations permit the very close juxtaposition of combining groups necessary for bonding. The hypothesis does not neglect the role of chemical composition in specificity, for the nature and arrangement of the residues will determine the type of folding possible at various points along the polypeptide chain. Nevertheless, chemical composition provides only the potentialities for specificity or a limited degree of specificity, the maximal degree being achieved by the folding process.

The possibility of applying the concept of complementariness to the more general problem of specificity in biological synthesis has been broached in several recent discussions (*4, 11, 13*). As Tyler (*12,* p. 13) has stated,

> Any of the macromolecular constituents synthesized in a cell would be complementary to the substances comprising the sites of synthesis. Since growth consists primarily in the formation of such substances that comprise the integral structure of the cell, we may regard the mechanism of the process of growth to be essentially analogous to that manifested in antibody formation.

The liver offers favorable material for the testing of this hypothesis, since the three broad elements in the chain of synthesis (nucleus, cytoplasm, cytoplasmic products) are readily available. If (1) nuclear constituents are the prime determiners of cytoplasmic activity, as ample evidence from classical and biochemical genetics would indicate, and (2) the cytoplasm is in turn a site of synthesis, then we might expect the complementary relationships diagramed in Fig. 1. Nuclear constituents (perhaps highly polymerized nucleohistones) serve as templates (N_1) for the synthesis of complementary cytoplasmic constituents, some of which act as templates (C_1) for the synthesis of cytoplasmic products (P_1), which in the present instance would be the serum proteins of hepatic origin. Omitting from consideration many obvious complicating factors (intermediate products, metabolic modifications, etc.), we should expect the serum products to have configurations resembling, although not perhaps exactly duplicating, the original nuclear templates. Furthermore, if antibodies contain configurations complementary to limited regions of the antigens, we may expect the general antibody-antigen relationships shown in Fig. 1. As indicated by the arrows, antinuclear bodies should react maximally with nuclear material and serum, while antiserum bodies should react maximally with nuclear and serum constituents. This is not what would be predicted on the basis of chemical composition; cytoplasm and serum certainly show a greater over-all chemical resemblance to one another than do nucleus and serum.

Rat liver nuclei and cytoplasm were separated by the Dounce method (*3*), using M/475 citric acid in the first step and distilled water at pH 5–7 thereafter. The injection of whole nuclei into rabbits indicated a low degree

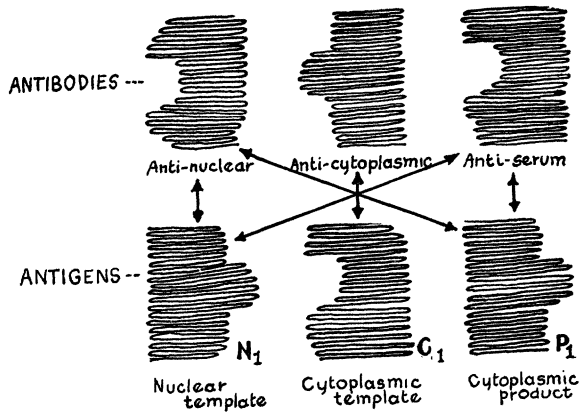

FIG. 1. Schema showing postulated complementary configurations of macromolecules of nucleus (N_1), cytoplasm (C_1), and cytoplasmic products (P_1). Portions of molecular chain represented by coiled lines. The arrows indicate expected maximal cross reactions between antibodies and antigens.

of antigenicity; hence the nuclei were treated with 10% NaCl solution to extract the nucleohistone (*9*) and make it available to the antibody-forming mechanism. All steps in the isolation and extraction procedures were carried out in the cold (6° C or below). The whole nuclei in 10% NaCl, alone or mixed with swine serum as adjuvant, were dialyzed first against water and then against physiological saline to remove excess salt and recombine the nucleic acid and histone. The products were injected intramuscularly and intraperitoneally into New Hampshire and White Leghorn fowl, and showed clear anti-