Yield was 11 g (75.9%). *Anal.* Calcd. for $C_7H_{15}O_2N$: C, 57.90; H, 10.42; N, 9.65. Found: C, 57.65; H, 10.45; N, 9.52.

Ethyl β-Piperidinopropionate——Prepared from 64 g (0.8 mole) of piperidine and 40 g (0.4 mole) of ethyl acrylate by the method of Adamson,[4a] bp 127—128°/32 mm. Yield was 63.5 g (88.2 %). *Anal.* Calcd. for $C_{10}H_{19}O_2N$: C, 64.83; H, 10.34; N, 7.56. Found: C, 64.96; H, 10.42; N, 7.69.

Polymethylenedimagnesium Dihalide——Magnesium turnings (0.2 atom) were covered with 20 ml of ether and a small amount of polymethylene dihalide was added and warmed to iniciate the reaction. If the reaction was not iniciated, a catalytic amount of iodine or ethyl bromide was added to the reaction mixture. As soon as the reaction was iniciated, the remainder of the halide (total 0.1 mole) was gradually added at such a rate that the mixture boils gently. Then the mixture was gently warmed until magnesium turnings were completely dissolved. The solution of polymethylenedimagnesium dihalide thus obtained was immediately used to the next reaction.

General Procedure for Synthesis of Cyclic Amino-*tert*-alcohol——To a ethereal solution of 0.1 mole of polymethylenedimagnesium dihalide prepared by the procedure described above, a solution of 0.025 mole of disubstituted amino acid ester was added at such a rate that the reaction mixture boils continuously. After addition, the mixture was gently refluxed for 30 min to complete the reaction, and then treated with 15% aqueous solution of HCl containing ice pieces. The aqueous layer was separated and made alkaline with aqueous NH_4OH solution. The separated oily product was extracted with ether, washed with H_2O and dried over Na_2SO_4. After removal of ether, the residue was purified by distilation under reduced pressure. Oxalic acid salt of piperidinomethylcyclopentan-1-ol was prepared by treating piperidinomethylcyclopentan-1-ol with the equimolar amount of oxalic acid in water. Prisms, mp 123—124°.

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Inhibition and Stimulation of the Biosynthesis of Protein and Nucleic Acid. IV.[1] Effect of 2-Amino-1,4-naphthoquinone Imine on the Biosynthesis of Nucleic Acid and Protein in Ehrlich Mouse Ascites Tumor Cells *in Vitro*

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In the previous papers dealing with the biological action of aminoquinone derivatives, it was shown that, among some derivatives, 2-amino-1,4-naphthoquinone imine-HCl (ANQI) had an intensive action to inhibit the synthesis of nucleic acid and protein, especially of DNA, in Ehrlich mouse ascites tumor cells *in vitro*,[1] and that this action was assumed to be attributable to its interacting activity with the moieties of purine bases in nucleic acid, particularly in native DNA.[3]

The present investigation was undertaken to clarify the details in the mode of inhibition of *in vitro* DNA, RNA, and protein synthesis in the tumor cells by ANQI. From the data described below, the following assumption may be possible; the inhibition of RNA and protein synthesis is not a primary action of ANQI, but a phenomenon being led secondarily by the inhibition of DNA synthesis.

1) Part III: S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 105 (1969).
2) Location; *Oshika, Shizuoka.*
3) S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 113 (1969).

Experimental

Materials——ANQI was synthesized in the author's laboratory according to the procedure of Fieser.[4] Deoxythymidine-2-^{14}C (TdR-2-^{14}C) and L-phenylalanine-U-^{14}C (L-Phe-U-^{14}C) were purchased from Daiichi Pure Chemicals Co., Ltd., and uridine-2-^{14}C (UR-2-^{14}C) was from The Radiochemical Centre. These radioactive compounds were used in 0.5—1.0 mCi/mmole of a specific radioactivity in the experiments on intact cells and 5 mCi/mmole in that on a cell-free system. Polyuridylic acid (poly U) was obtained from Sigma Chemical Co. Pancreatic deoxyribonuclease (DNase) was the product of Worthington Biochemical Corp.

Ehrlich Mouse Ascites Tumor Cells——Preparation of the cells was carried out as described earlier.[5]

Incubation and Fractionation Procedures in the Intact Tumor Cells——A mixture (1.5 ml) consisted of the intact tumor cells (*ca.* 1×10^8), a radioactive precursor (1×10^{-4}M), and ANQI in Ca^{2+}-free modified Krebs-Ringer phosphate buffer (pH 7.2)[6] was incubated at 37° as described in the previous paper.[1] In the experiment on the pre-treatment of the tumor cells with ANQI, the mixture without the precursor was first incubated at 37° for 10 min, then the precursor was added to the mixture and incubated at 37° for 60min. The fractionation of DNA and RNA was carried out according to the method of Schmidt, Thannhauser, and Schneider,[7] and of protein was performed as described earlier.[5]

Amino Acid Incorporation in a Cell-Free System——The fractionation of subcellular fractions and the incubation procedure were essentially followed to the methods of Littlefield and Keller,[8] and of Rendi and Ochoa.[9] The incubation mixture contained in micromoles in a final volume of 1.5 ml: KCl, 300; $MgCl_2$, 15; Tris-HCl buffer, pH 7.8, 150; ATP, 0.75; GTP, 0.12; phosphocreatine, 20; phosphocreatine kinase, 75 μg; L-Phe-U-^{14}C, 0.15 (final concentration, 1×10^{-4}M); mercaptoethanol, 120; poly U, 10 μg; DNase, 10 μg; ANQI, as indicated; and post-mitochondrial supernatant fraction ($15000\times g$, 10 min) of the tumor cells in the medium described by Littlefield and Keller,[8] 0.5 ml.

After incubation for 60 min at 37°, the reaction was stopped by the addition of 0.5 ml of cold 20% trichloroacetic acid (TCA), and the precipitate was washed with cold 5% TCA (3 ml) and with a mixture (3 ml) of ethanol, ether, and chloroform (2:2:1), then treated with 2 ml of 1N NaOH for 60 min at 37° for removal of the radioactive amino acid from aminoacyl-t-RNA, and neutralized with 6N HCl. The suspension was extracted with 5% TCA (2.5 ml) at 100° for 15 min to remove nucleic acid, then the residual protein was washed with cold 5% TCA (3 ml), with acetone (3 ml, twice), and plated on aluminum disks.

Assay for Radioactivities and Incorporation of Precursor——The radioactivities were assayed in a windowless 2π-gas-flow counter as described earlier.[1] The amounts of incorporated precursors were calculated also as reported in the previous paper.[1]

Results and Discussion

Time Course of Inhibition of DNA, RNA, and Protein Synthesis by ANQI

When the tumor cells were incubated at 37° with TdR-2-^{14}C, UR-2-^{14}C, or L-Phe-U-^{14}C (1×10^{-4}M, each) in the presence of 2×10^{-6}M of ANQI, the incorporation of each precursor into DNA, RNA, or protein, respectively, was inhibited to various degree as presented in Fig. 1. The time course of each incorporation shows that there were some differences in the rates of inhibition among these three cases. That is, the incorporation of TdR-2-^{14}C into DNA was most inhibited by ANQI, and to nearly the same extent at each incubation time, at least in the period of 5—180 min, whereas the extents of inhibition of UR-2-^{14}C and L-Phe-U-^{14}C incorporation into RNA and protein, respectively, were increased with a passage of incubation time as shown in Fig. 2, in which were summarized the inhibition rates in Fig. 1.

These observations suggest that the inhibition of RNA and protein synthesis was caused with a delay to that of DNA synthesis, and was not a primary action of ANQI but a secondary one being led by the inhibition of DNA synthesis.

4) L.F. Fieser, "Experiments in Organic Chemistry," 3rd ed., D.C. Heath & Co., Boston, 1957 (reprinted by Maruzen Co., Ltd., Tokyo, 1958), pp. 234—238.
5) O. Tamemasa, S. Okada, and Y. Wakita, *Chem. Pharm. Bull.* (Tokyo), **13**, 1193 (1965).
6) M. Rabinovitz, M.E. Olson, and D.M. Greenberg, *J. Biol. Chem.*, **210**, 837 (1954).
7) G. Schmidt and S.J. Thannhauser, *J. Biol. Chem.*, **161**, 83 (1945); W.C. Schneider, *ibid.*, **161**, 293 (1945); W.C. Schneider, *ibid.*, **164**, 747 (1946); S. Mizuno, *Kagaku To Seibutsu*, **3**, 148 (1965).
8) J.W. Littlefield and E.B. Keller, *J. Biol. Chem.*, **224**, 13 (1957).
9) R. Rendi and S. Ochoa, *J. Biol. Chem.*, **237**, 3707 (1962).

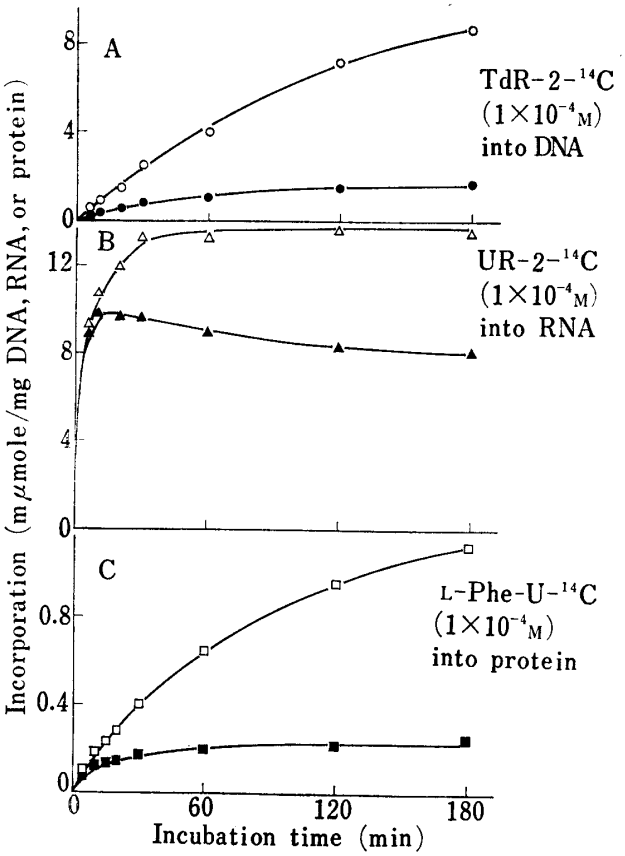

Fig. 1. Time Course of Incorporation of TdR-2-^{14}C (A), UR-2-^{14}C (B), and L-Phe-U-^{14}C (C) into DNA, RNA, and Protein, respectively, in the Presence of ANQI (2×10^{-6}M)

—○— —△— —□—: control
—●— —▲— —■—: ANQI

On the other hand, it is of interest that in the presence of ANQI the incorporation of UR-2-^{14}C was gradually decreased from 10 min after starting the incubation. This phenomenon may be explained from the fact that the degradation of RNA was stimulated by ANQI to some extent.[1)]

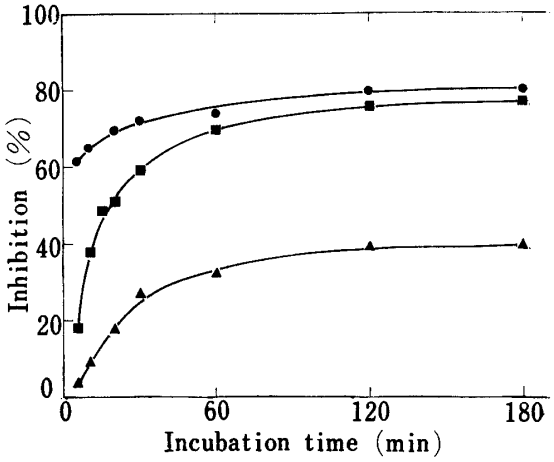

Fig. 2. Time Course of Inhibition of TdR-2-^{14}C, UR-2-^{14}C, and L-Phe-U-^{14}C Incorporation into DNA, RNA, and Protein, respectively, by ANQI (2×10^{-6}M)

—●—: TdR-2-^{14}C
—▲—: UR-2-^{14}C } (1×10^{-4}M) into {DNA, RNA
—■—: L-Phe-U-^{14}C } {protein

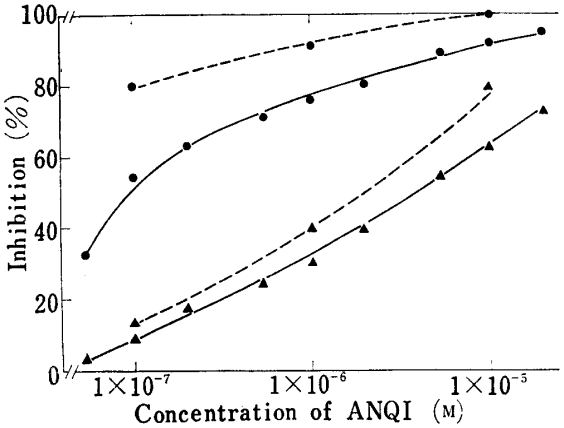

Fig. 3. Effect of ANQI on TdR-2-^{14}C and UR-2-^{14}C Incorporation into DNA and RNA, respectively

●: TdR-2-^{14}C
▲: UR-2-^{14}C } (1×10^{-4}M) into {DNA, RNA
——: Simultaneous incubation; the cells were incubated simultaneously with ANQI and a precursor at 37° for 60 min.
——: Pre-incubation; the cells were incubated previously with ANQI at 37° for 10 min, then a precursor was added and re-incubated at 37° for 60 min.

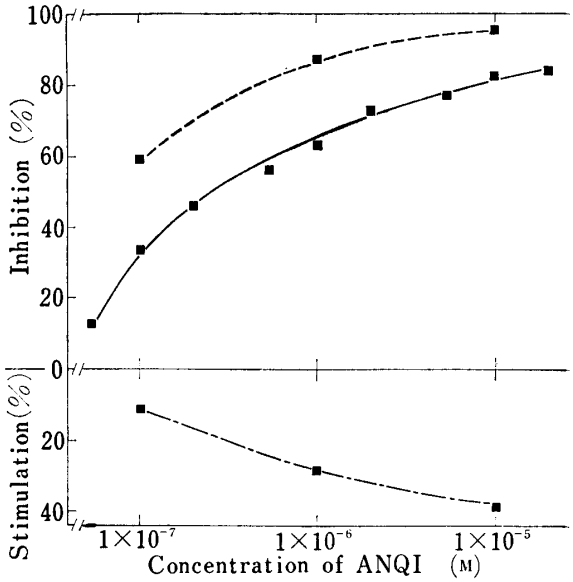

Fig. 4. Effect of ANQI on L-Phe-U-^{14}C (1×10^{-4}M) Incorporation into Protein

——: simultaneous incubation; the same as in Fig. 3
------: pre-incubation; the same as in Fig. 3
– – –: cell-free system; see in the text

DNA, RNA, and Protein Synthesis in the Tumor Cells Pre-Treated with ANQI

When the tumor cells were incubated with ANQI at 37° for 10 min prior to the addition of a precursor (TdR-2-^{14}C, UR-2-^{14}C, or L-Phe-U-^{14}C) at a final concentration of 1×10^{-4}M, the incorporation of each precursor was inhibited to a greater extent than that in the experiment of simultaneous incubation (Fig. 3 and 4).[10] The incorporation of TdR-2-^{14}C into DNA was most intensively inhibited by such a method of incubation, that is, the extent of inhibition was nearly 100% at 1×10^{-5}M of ANQI and was approximately 90% at 1×10^{-6}M, and each extent at each concentration of ANQI in the experiment of pre-incubation corresponded to that at 10 times or moreover higher concentration of ANQI in the experiment of simultaneous incubation.

On the other hand, as to the increases in the extents of inhibition upon the incorporation of UR-2-^{14}C into RNA and L-Phe-U-^{14}C into protein by the method of pre-incubation, it was found that the former was a little and lesser to but the latter was great and comparable to that in the case of TdR-2-^{14}C incorporation into DNA.

The reason for the increases in the extents of inhibition by the pre-incubation is not clear, for the data on the intact cells include a lot of complex factors. However, these observations are, at least, not in conflict with the assumption made from the data in Fig. 1 and 2, *i.e.*, the primary action of ANQI is the inhibition of DNA synthesis. That is, the interaction between ANQI and DNA in the tumor cells, if it is produced equally with the *in vitro* experiment,[3] is considered to become more complete by the pre-incubation, and consequently, DNA synthesis is inhibited more intensively than in the experiment of simultaneous incubation. Furthermore, the more intensive inhibition of RNA and protein synthesis is also considered to be derived from that of DNA synthesis.

Effect on Polypeptide Synthesis in a Cell-Free System

In order to confirm the assumption mentioned above the effect of ANQI was examined on the incorporation of L-Phe-U-^{14}C into polyphenylalanine in a cell-free system contained poly U and DNase. As seen in Fig. 4, the incorporation of L-Phe-U-^{14}C was not inhibited, but rather stimulated by ANQI in this system. This finding leads to a conclusion that the inhibition of protein synthesis is not caused without the inhibition of DNA synthesis by ANQI and is evidently a secondary action of ANQI, though the reason for the stimulation of L-Phe-U-^{14}C incorporation in the cell-free system is not clear. The following two observations also make this conclusion reasonable; ANQI did not interact with poly U,[3] and in a preliminary experiment ANQI was not reduced by mercaptoethanol which was a component of the incubation mixture of cell-free system.

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10) The data of simultaneous incubation in Fig. 3 and 4 are cited from the previous paper[1] for reference.