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An acute effect of high oxygen tension on the uptake of ^{3}H-deoxycytidine into thymocyte deoxyribonucleic acid

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IT IS GENERALLY believed that use of oxygen tensions of greater than 30 per cent in mammalian cell cultures will inhibit growth and nucleic acid synthesis.[1] Andersen *et al.*,[2] using human lymphocytes stimulated with phytohemagglutinin, showed that an atmosphere of 5–20% O_2 gave optimal rates of incorporation of ^{3}H-thymidine in 68-hr cultures. After 48 hr of culture employing 80% O_2, myocardial cells displayed depressed ^{14}C-uridine incorporation compared to cells grown under 20% O_2.[3] However, little data are available concerning the acute effects of high oxygen tension on nucleic acid metabolism in mammalian cells.

Thymic lymphocyte suspensions were prepared from 2 to 4 Carworth Farm rats that had been maintained on Rockland Lab Chow and tap water *ad lib.* for at least 1 week. The animals were sacrificed by decapitation, and the thymuses were placed in ice-cold minimal essential medium (MEM Joklik modified, Grand Island Co., New York) that had been gassed previously with 90% air–10% CO_2 or 90% O_2–10% CO_2 to bring the pH to 7·0–7·2. Cells were teased out of the thymus tissue, and the suspension was filtered through a 250 mesh stainless steel screen to remove the connective tissue. The thymocytes were washed three times at 0–4° with MEM using centrifugation at 600 *g* for 2 min. The cell suspension was made up to 3·0 ml and counted by standard hematological techniques.

Cultures of 1·5 ml containing 4·0 × 10^7 cells/ml were gassed, capped and preincubated in duplicate for 0–3 hr at 37°. At the appropriate time, 0·5 ml of each cell suspension was pulsed in duplicate under the same gas for 1 hr with 1 μCi of a ^{3}H-nucleoside in 0·1 ml MEM. Incorporation of the radioactivity was terminated by the rapid addition of ice-cold MEM containing 1 × 10^{-4} M non-radioactive nucleoside. The cells were centrifuged, the supernatant was aspirated, and the cell button washed with 5% trichloroacetic acid (TCA) on a nitro cellulose filter (Schleicher & Schuell, average pore size 0·45 μm). The filters were dried under a heat lamp and placed in 15 ml scintillation fluid[4] for at least 18 hr prior to counting in a Nuclear–Chicago Unilux II liquid scintillation counter. Quenching was determined to be essentially constant for all tubes; therefore, results are reported in cpm for the 2·0 × 10^7 cells counted. All glassware which came into contact with the cells during incubation or labeling was siliconized prior to use and washed in 7X nonionic detergent.

Radioisotopes were purchased from Schwarz Bioresearch, Inc. and had specific activities of 26·2, 3·0 and 2·0 Ci/m-mole for deoxycytidine-5-^{3}H, thymidine-methyl-^{3}H and uridine-5-^{3}H respectively.

Figure 1 shows the effect of high and low oxygen tensions on the relative rates of incorporation of ^{3}H-nucleosides into nucleic acids. Incorporation rates are compared to those obtained without preincubation. Using 90% O_2-10% CO_2 as the gas phase, uptake of ^{3}H-deoxycytidine into DNA declined rapidly over

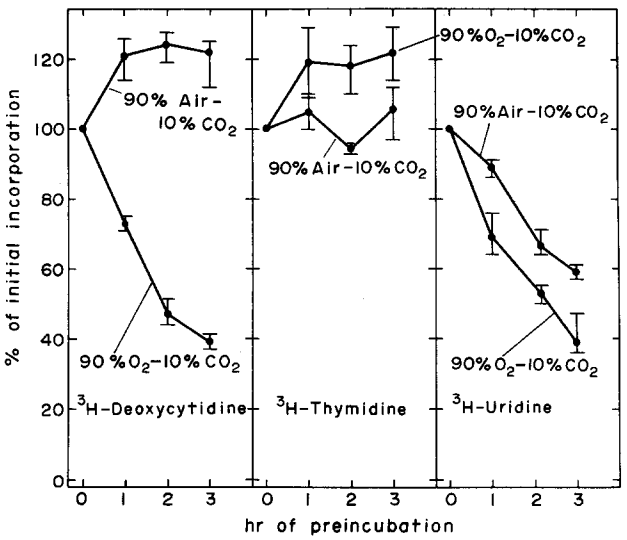

FIG. 1. Incorporation of ^{3}H-nucleosides into nucleic acids of rat thymic lymphocytes in 90% air–10% CO_2 and 90% O_2–10% CO_2 atmospheres. The cells were preincubated for the times shown, and then pulsed for 1 hr with the appropriate ^{3}H-nucleoside in the same gas phase. The results are expressed as a percentage of the average incorporation without preincubation. Preincubation and the pulses were in duplicate. Vertical lines represent the range of percentages for individual tubes compared to the initial averages which were 8,230, 9,970 and 4,860 cpm in the 90% air–10% CO_2, and 7,140, 10,790 and 2,460 cpm in the 90% O_2–10% CO_2 for ^{3}H-deoxycytidine, ^{3}H-thymidine and ^{3}H-uridine.

the 3-hr period. In contrast, the incorporation of ^{3}H-thymidine into DNA in this gas phase was maintained. On the other hand, cells incubated in the 90% air–10% CO_2 atmosphere showed small increases in their rates of uptake of both ^{3}H-deoxycytidine and ^{3}H-thymidine into DNA. The incorporation of ^{3}H-uridine into nucleic acids declined in both atmospheres.

Since the uptake of ^{3}H-thymidine into DNA was not suppressed in the high oxygen tension, the pronounced inhibitory effect on the incorporation of ^{3}H-deoxycytidine into DNA in this atmosphere cannot be due to either inhibition of DNA synthesis or a general killing of the cells. An explanation for the oxygen-induced decline of ^{3}H-deoxycytidine uptake into DNA might be found in an increased degradation of the precursor by the cells, or decreased transport and/or phosphorylation of the ^{3}H-deoxycytidine. It has been shown, using calf thymus enzymes, that both deoxycytidine kinase[5] and dCMP kinase[6] are activated by sulfhydryl compounds, suggesting that free sulfhydryls are necessary for their activity. Some of the toxic effects of oxygen have been postulated to be exerted through oxidation of sulfhydryl groups on proteins.[1,7] An inhibitory effect on either of these kinases would suppress the incorporation of ^{3}H-deoxycytidine into DNA but would still permit the synthesis of dCTP by the pathway CDP → dCDP → dCTP, thus causing a decrease in the specific activity of the dCTP pool. The enzyme ribonucleotide reductase and thioredoxin constitute an important sulfhydryl system that also could be influenced by oxygen.[8] Since ^{3}H-deoxycytidine is incorporated into thymine in DNA, as well as cytosine,[9] an effect of oxygen resulting in an inhibition of the enzyme dCMP deaminase, which is essential for synthesis of dTTP from dCMP, could also decrease uptake of ^{3}H-deoxycytidine into DNA.

The results presented indicate that there is an acute effect of high oxygen tension on nucleic acid metabolism in thymocytes which is manifested by a decline in ^{3}H-deoxycytidine incorporation into DNA. This effect may serve as a model for studies concerning the effect of oxygen on a biological system.

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Effect of 1-(2-carboxy-3-chlorophenyl)pyrrole on carbohydrate metabolism in rat and mouse

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IN THE COURSE of screening for novel hypoglycemic agents, a series of 1-(2-carboxyphenyl)pyrroles and their cyclized derivatives, pyrrolo-(1,2-a)indoles, were found to have a hypoglycemic action in the rat (unpublished data). Among these compounds, 1-(2-carboxy-3-chlorophenyl)-pyrrole (T-9078) was the most active. The present report describes our biochemical studies on the mode of action of the hypoglycemics and presents evidence that the hypoglycemia is caused by a stimulation of glucose utilization in peripheral tissues.

Materials. Male SD rats and ICR mice were purchased from CLEA (Tokyo, Japan), fed a stock diet, and were used at 5–6 weeks of age. Test kits for enzyme analysis of D-glucose, L-lactate and pyruvate were obtained from Boehringer Mannheim Corp.; D-(U-^{14}C)glucose (309 mCi/m-mole), L-(U-^{14}C)alanine (173 mCi/m-mole) and insulin immunoassay kit were obtained from the Radiochemical Centre, England. Ion-exchange resins, AG 1 × 8 (acetate) and AG 50 × 4 (H), were purchased from BioRad Lab., and bovine albumin fraction V and 2-deoxyglucose from Wako Chem. Ind. Ltd. 1-(2-Carboxy-3-chlorophenyl)pyrrole was synthesized and donated for the present studies by Dr. Y. Kawamatsu of the Chemical Research Laboratories of our Division.

Methods. Blood glucose was measured by the glucose oxidase method (Test kit) as modified to fit the Autolab autoanalyser (Linson Inst. AB, Stockholm). Glycogen was determined by the anthrone method.[1]

Eviscerated, nephrectomized rats. Evisceration was performed as described by Russell.[2] The kidneys were functionally removed by ligature of the renal arteries and veins. Operated rats received 400 mg/kg of glucose subcutaneously at 1-hr intervals. The test compound was administered subcutaneously immediately after the operation. Blood samples were taken from the tail vein 3 hr after administration of the compound.

(U-^{14}C)glucose oxidation in intact mice. Fasted mice received intraperitoneal injections of the test compound and tracer doses of (U-^{14}C)glucose (2 μCi) and were placed in metabolic cages. The expired carbon-14 was continuously measured by an ionization chamber (Cary Instruments), the CO_2 excretion by infrared absorption (Horiba Ltd., Kyoto), and the specific radioactivity by a radioanalyzer (The Rikadenki Ltd.) as described by Tolbert *et al.*[3]

Uptake of 2-deoxyglucose by intact diaphragm. Intact diaphragm was taken from rats pretreated with the drug (T-9078) and incubated for 1 hr at 37° in 50 ml of Krebs–Ringer bicarbonate buffer, pH 7·4, containing 20 mM 2-deoxyglucose. The hemidiaphragms were then excised and the content of total 2-deoxyglucose and 2-deoxyglucose-6-phosphate was determined.[4]

Gluconeogenesis in vivo. Rats were injected intraperitoneally with a tracer dose of (U-^{14}C)alanine (10 μCi/rat). Blood samples were taken from the tail vein 30 min after the injection. Radioglucose was separated from radioactive ionic materials in deproteinized blood by passage through a column consisting of mixed-bed (AG 1 × 8 and AG 50 × 4) ion-exchange resins. Radioactivity was determined in a Packard liquid scintillation spectrometer.