Effect of Spontaneous Mammary Carcinogenesis on *in vivo* Glucose-U-C^{14} Incorporation in C3H Mice.* (32303)

KARAM VIR BATRA† AND GEORGE T. OKITA (Introduced by J. A. Wells)
Argonne Cancer Research Hospital‡ and Department of Pharmacology, University of Chicago, and Department of Pharmacology, Northwestern University Medical School, Chicago, Ill.

In an earlier communication on the *in vivo* oxidation of C^{14}-labeled carbohydrate intermediates we reported differences between control *versus* precancerous and cancerous C3H mice(1). A significant increase in glucose-6-C^{14} oxidation and a decrease in glucose-1-C^{14} oxidation to $C^{14}O_2$ were observed in both precancerous and mammary tumor-bearing C3H mice. In addition, a decrease in the oxidation of citrate-1,5-C^{14} was noted in the same mice in comparison to controls.

Therefore, previous findings suggest an increase in *in vivo* glycolytic metabolism and a decrease in the pentose phosphate pathway and the Krebs citric acid cycle during mammary oncogenesis. In an attempt to obtain additional insight on the observed biochemical differences between carcinogenic and non-carcinogenic mice, studies were conducted to determine the effect of mammary carcinogenesis on the incorporation of glucose-U-C^{14} into selected biochemical intermediates.

Materials and method. The animals used were inbred C3H/He mice (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine), which have a high genetic susceptibility (>95%) to spontaneous mammary tumor(2). Mice bearing transplanted tumors were not used in order to avoid biochemical artifacts that can arise as a result of immunological responses(3). Healthy female C3H/He mice at 3 different stages of development were used: (a) sexually mature young virgins between 3 and 4 months of age (average weight 22 g), (b) multiparous exbreeders without tumors but considered to be in the precancerous stage because of the development of precancerous lesions in the mammary gland(4) (average weight 28 g), and (c) multiparous exbreeders with a single spontaneous mammary tumor not exceeding 1.5 cm in diameter (average weight 30 g). All mice were maintained on standard Rockland mouse diet in air-conditioned animal quarters at a temperature of approximately 25°C. The mice were fasted between 10 to 14 hr prior to the study but given free access to water at all times.

Tracer amounts of glucose-U-C^{14} (72.9 millicurie/millimole, Nuclear Chicago Corp.) of high specific activity were administered intraperitoneally in doses of 0.2 microcurie (0.5 μg) per gram of body weight. The mice were sacrificed 2, 6, and 12 minutes later by immersion in liquid nitrogen. The livers and tumors were removed while still in the frozen state, cleared of undesired tissues, blotted dry, and kept on ice. Organs from at least 3 mice were pooled and used for each assay. Homogenates were prepared with perchloric acid as described by Busch *et al*(5), and were brought to pH 7.3 $\pm$ 0.1 with 2 *N* potassium hydroxide. Supernates were obtained for subsequent fractionation of various intermediates.

For the fractional separation of labeled intermediates, glutamic, aspartic, and lactic acids were isolated by using a Dowex 1-X8 200 to 400 mesh resin column in the acetate form with a gradient elution of 0 to 4 N acetic acid. The initial water washings of the column represented the neutral fractions that contained glucose. Three-ml fractions were collected at a rate of 15 to 20 drops/min. Glutamic, aspartic, and lactic acids appeared approximately in tube numbers 20, 30, and 55, respectively.

For the isolation of labeled glucose, the water washing from the resin column termed "neutral effluent" was desalted with a cationic resin, Dowex 50-X4, 20 to 50 mesh. An aliquot from the desalted solution was chro-

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† Present address: I. I. T. Research Institute Chicago, Ill.

‡ Operated by University of Chicago for Atomic Energy Commission.

matographed on Whatman No. 1 paper in parallel with a standard glucose sample for 40 to 48 hr by using an n-butanol:water:ethyl alcohol (4:5:1) solvent system. The standard glucose strip was sprayed with 10% aqueous molybdate solution and heated in an oven for 20 min at 100°C to indicate the location of glucose. This strip was used as a reference marker, and confirmation was made by a radiochromatogram scan of the sample strip. The radioactivve band containing glucose was cut out and eluted by centrifugation at low speed with a minimum amount of water. The eluted aqueous solution was rechromatographed, and the whole procedure repeated to obtain maximum purity of the isolated glucose.

The various isolated intermediates were confirmed and checked for purity by paper chromatography as follows: amino acids by 2-dimensional chromatography(6) and lactic acid(6,7) and alpha-ketoglutaric acid(6,8,9) by one-dimensional paper chromatography. At least 2 different solvent systems were used for each intermediate.

For quantitative determination of intermediates, glucose was assayed enzymatically by the method of Keilin and Hartree(10), in which glucose-oxidase, peroxidase, and O-dianisidine are used. Amino acids were assayed by the photometric ninhydrin method (11,12). Lactic acid was assayed by the method of Barker and Summerson(13), which is based upon formation of acetaldehyde from lactic acid by treatment with sulfuric acid and subsequent development of a purple color with parahydroxydiphenyl. Alpha-ketoglutaric acid was assayed by the method of Katsuki *et al*(14). By utilizing this method, a 2,4-dinitrophenylhydrazine derivative of alpha-ketoglutaric acid was isolated and assayed both for amount and radioactivity.

For radioassay of C^{14}-labeled compounds, a liquid scintillation spectrometer (Tri-Carb, Packard Instrument Corp.) was used. All samples were counted in an aqueous scintillator solvent system(15).

Results and discussion. To account for previously noted(1) differences in *in vivo* oxidation of C^{14}-labeled glucose between precancerous and mammary tumor-bearing C3H mice *versus* controls, the effect of spontaneous mammary carcinogenesis on the rate of incorporation of glucose-U-C^{14} into various biochemical intermediates was investigated. Although it would have been desirable to compare data between intermediates isolated from mammary tumors and from normal mammary tissue, this was not possible because of the difficulty of obtaining suitable amounts of normal mammary gland tissue of mice. Therefore, intermediates isolated from livers during various stages of carcinogenesis were compared.

Our earlier *in vivo* studies(1) on the rates of oxidation of various C^{14}-labeled intermediates to $C^{14}O_2$ indicated that the altered metabolism noted in precancerous and tumorous mice represented host changes in total body metabolism. This conclusion is based on our finding that an increase of approximately 40% in the *in vivo* oxidation of glucose-6-C^{14} to $C^{14}O_2$ occurred in tumor-bearing mice in comparison with controls, whereas the average increase in body weight due to the tumor mass was less than 3%.

Labeled intermediates isolated from liver and tumor were also compared. Although tumor and liver are morphologically different, they were compared on the basis that both are actively metabolizing organs, as indicated by the rapid growth rate of tumors and the role of liver as a major metabolic organ.

Effect of carcinogenesis on tissue glucose. To interpret properly the metabolic effects of carcinogenesis on glucose-U-C^{14} incorporation, it is necessary to determine initially the kinetics of the parent compound, glucose. By isolating labeled glucose from tissues at various time intervals after glucose-U-C^{14} administration, relative differences in tissue glucose concentration, pool size, turnover rate, and rate of incorporation into other metabolic products can be determined. The recovery of labeled glucose after intraperitoneal administration of glucose-U-C^{14} reflects the rapid glucose equilibration that occurred during the first few minutes, as indicated by the initial build-up phase of the specific activity curve. This rapid equilibration was followed by dilution of specific activity resulting from synthesis and turnover after the peak time of glu-

TABLE I. Effect of Various Stages of Carcinogenesis on Mean Specific Activity and Tissue Concentration of Glucose After Glucose-U-C^{14} Administration to C3H Mice.

Experimental animals	Tissue	Mean specific activity of recovered glucose, DPM/μg ± S.D. 2 min	6 min	12 min	Concentration, μg/g tissue ± S.D.	Tissue pool, μg/organ ± S.D.
Virgin controls	Liver	170 ± 100	275 ± 95	300 ± 200	3800 ± 750	3290 ± 690
Precancerous exbreeders	Liver	210 ± 130	265 ± 14	330 ± 255	4100 ±1100	4700 ± 1365 P <.01
Tumor exbreeders	Liver	245[a] ± 46	370[b] ±150	130 ± 70	4500 ± 460	5600 ± 2025 P <.005
	Mammary tumor	140[a] ± 20	380[c] ± 30	650 ± 90	330 ± 150	135 ± 100
					P <.005 P_1<.005	P <.005 P_1<.005

P = difference between experimental animals and virgin controls.
P_1 = difference between tumors and livers of tumorous animals.
N = 3, except where otherwise indicated. Each observation represents assay of organs pooled from at least 3 animals.
[a] N = 4. [b] N = 5. [c] Mean deviation of 2 values.

cose specific activity was passed.

Table I shows the effect of various stages of carcinogenesis on mean specific activity (DPM/μg), tissue concentration (μg/g of wet tissue), and tissue pool (μg/organ) of glucose after administration of glucose-U-C^{14} and sacrifice of the animal. There was no difference in the liver glucose concentration in control mice as compared with precancerous and tumor-bearing mice. Also, there was no difference in equilibration between administered labeled glucose and liver glucose, as indicated by similarities in the specific activities of the isolated glucose at 2 min. However, the lower specific activity of liver glucose at 12 min following peak values at 6 minutes indicates an increase in synthesis and turnover of glucose in tumor-bearing mice in comparison with control and precancerous C3H mice.

The concentration of glucose in mammary tumor tissue was approximately 1/13th the concentration of liver glucose in tumor-bearing mice. In spite of the lower tumor glucose concentration, its specific activity at 2 minutes was lower than that of liver glucose, suggesting a slower mixing and turnover time for tumor glucose. This was further indicated by the continued increase in the specific activity of tumor glucose at the 12-minute interval in comparison with that of liver glucose. The lack of a fall in the specific activity of tumor glucose at this time period suggests that it has lower synthesis and turnover rates than liver glucose.

Effect of carcinogenesis on glucose-U-C^{14} incorporation. To determine the effects of the cancerous and precancerous state on *in vivo* intermediary carbohydrate metabolism, the incorporation of glucose-U-C^{14} into various glycolytic and Krebs cycle intermediates was investigated. Lactic acid was one of the principal intermediates considered, since it is an important product of glycolytic metabolism.

Table II presents data on the incorporation of glucose-U-C^{14} into liver and tumor lactic acid. For liver lactic acid, there were no appreciable differences in tissue concentration or specific activity among the various experimental groups of mice. However, in tumor-bearing mice, the tumor lactic acid concentration was elevated in comparison with the liver lactic acid concentration. This increase agrees with reports for most types of tumor(16,17,18). In comparing the rates of incorporation of glucose-U-C^{14} into tumor and liver lactate of tumor-bearing mice, there was an apparent increase in incorporation into tumor lactate, as suggested by the slight increase in specific activity of tumor lactate at 12 minutes in spite of a higher tissue concentration. However, because of the 5-fold increase in specific activity of tumor glucose over liver glucose at 12 minutes the increase in tumor lactate specific activity is not believed to be significant. Therefore, the results suggest that there is no increase in the rate of synthess of lactic acid in tumor over liver. The higher concentration of tumor lactate

TABLE II. Effect of Carcinogenesis on Incorporation of Glucose-U-C^{14} into Lactic Acid.

Experimental animals	Tissue	Mean specific activity of recovered lactate, DPM/μg ± S.D. 2 min	6 min	12 min	Concentration, μg/g tissue ± S.D.
Virgin controls	Liver	28 ± 9	54 ± 5	80 ± 17	440 ± 110
Precancerous exbreeders	Liver	24 ± 4	61 ± 4	90 ± 10	340 ± 70
Tumor exbreeders	Liver	25[a] ± 4	68[b] ± 8	81 ± 12	370 ± 110
	Mammary tumor	8.8[a] ± 2	66[b] ± 18	100 ± 12	580 ± 140
					P <.025
					P_1 <.005

P = difference between experimental animals and virgin controls.
P_1 = difference between tumors and livers of tumorous animals.
N = 3, except where otherwise indicated. Each observation represents assay of organs pooled from at least 3 animals.
[a] N = 4. [b] N = 5.

TABLE III. Effect of Carcinogenesis on Incorporation of Glucose-U-C^{14} into α-Ketoglutaric Acid.

Experimental animals	Tissue	Mean specific activity of recovered α-KG at 6 min, DPM/μg ± S.D.	Concentration, μg/g tissue ± S.D.
Virgin controls	Liver	30 ± 11	12 ± 3
Precancerous exbreeders	Liver	38 ± 9	12 ± 2
Tumor exbreeders	Liver	55[a] ± 28	13 ± 3
	Mammary tumor	42[a] ± 37	22 ±13
			P_1 <.1

P_1 = difference between tumors and livers of tumorous animals.
N = 3, except where otherwise indicated. Each observation represents assay of organs pooled from at least 3 animals.
[a] N = 4.

over liver lactate may be due to a slower rate of catabolism of tumor lactic acid.

An important intermediate in the oxidation of glucose *via* the Krebs cycle is alpha-ketoglutaric acid (α-KG). Alteration in its metabolism may indicate whether carcinogenesis has any influence on the operation of the cycle. Table III presents data on the incorporation of glucose-U-C^{14} into α-KG 6 minutes after administration of the labeled intermediate. No significant differences were observed in changes in specific activities of α-KG isolated from livers or tumors of various experimental groups. For tumor α-KG an increase in concentration was noted in comparison with liver α-KG. The rise in α-KG concentration with no change in specific activity may indicate a decrease in tumor α-KG catabolism. This finding is compatible with our previously reported data(1), in which a decrease in citrate-1,5-C^{14} oxidation was noted in tumor-bearing C3H mice, suggesting a decrease in activity of the Krebs cycle.

The incorporation of glucose-U-C^{14} into glutamic and aspartic acid was also investigated. As shown in Table IV, a decrease in specific activity and an increase in glutamate tissue concentration was observed for tumor glutamic acid. The results therefore indicate an inhibition in both synthesis and catabolism; the decrease in catabolism was greater than the decrease in synthesis. Since glutamic acid is in equilibrium with α-KG, this further confirms the reduction of Krebs cycle activity noted with α-KG. For livers of the various experimental groups, there were no significant differences in the rate of incorporation of glucose-U-C^{14} into liver glutamate.

Results on the incorporation of labeled glucose into aspartate are given in Table V. An increase in liver aspartic acid specific activity for precancerous and tumorous animals was observed. However, for tumor aspartate there was a decrease in specific activity and no change in tissue levels when compared

TABLE IV. Effect of Carcinogenesis on Incorporation of Glucose-U-C^{14} into Glutamic Acid.

Experimental animals	Tissue	Mean specific activity of recovered glutamate, DPM/μg ± S.D. 2 min	6 min	12 min	Concentration, μg/g tissue ± S.D.
Virgin controls	Liver	3.5 ± 1	8 ± 2.6	20 ± 9	301 ± 110
Precancerous exbreeders	Liver	4 ± 1	9 ± 1	31 ± 2.6	301 ± 80
Tumor exbreeders	Liver	3.7[a] ± .4	11[b] ± 1.2	23 ± 2.5	320 ± 45
	Mammary tumor	.8[a] ± .8	1.6[b] ± .5	5.4 ± 2.5	520 ± 60
					P <.005
					P_1 <.005

P = difference between experimental animals and virgin controls.
P_1 = difference between tumors and livers of tumorous animals.
N = 3, except where otherwise indicated. Each observation represents assay of organs pooled from at least 3 animals.
[a] N = 4. [b] N = 5.

TABLE V. Effect of Carcinogenesis on the Incorporation of Glucose-U-C^{14} into Aspartic Acid.

Experimental animals	Tissue	Mean specific activity of recovered aspartate, DPM/μg ± S.D. 2 min	6 min	12 min	Concentration, μg/g tissue ± S.D.
Virgin controls	Liver	4.8 ± 1.9	6.6 ± 3.9	8.4 ± 2.8	180 ± 80
Precancerous exbreeders	Liver	4.1 ± 2.5	14.6 ± .2	30 ± 5	140 ± 70
Tumor exbreeders	Liver	5.4[a] ± 2.7	12[b] ± 5	24 ± 3.4	175 ± 90
	Mammary tumor	.4[a] ± .5	3.9[b] ± 2.7	7.14 ± 1.9	190 ± 60

N = 3, except where otherwise indicated. Each observation represents assay of organs pooled from at least 3 animals.
[a] N = 4. [b] N = 5.

to liver aspartic acid, indicating a reduced rate of synthesis and catabolism. Therefore, the reduction in tumor aspartic acid synthesis and turnover also suggests a decrease in the Krebs cycle in tumors, since aspartic acid is in equilibrium with oxaloacetate.

This *in vivo* study therefore indicates that spontaneous mammary tumors of C3H mice demonstrate a decrease in oxidative metabolism *via* the Krebs cycle, in comparison with liver. These findings are consistent with *in vitro* results of others, such as Warburg(19), Burk *et al*(20), Dickens and Weil-Malherbe (21), Meyerhof(22), and LePage(23), who also reported decreases in oxidative metabolism for various types of tumors. With respect to glycolytic metabolism of mammary tumor tissue, no differences were observed in comparison with liver.

In comparing the incorporation of glucose-U-C^{14} into livers of the various experimental groups, no consistent differences were noted in both glycolytic and oxidative metabolism between control, precancerous, and tumor-bearing mice. Therefore, the metabolic differences noted in our previous *in vivo* studies may be due to differences in extrahepatic metabolism as influenced by the precancerous and cancer state.

Summary. Studies were conducted to elucidate previously observed differences between carcinogenic and noncarcinogenic mice. Glucose-U-C^{14} was incorporated into biochemical intermediates (lactic acid, glutamic acid, aspartic acid, and alpha-ketoglutaric acid) in C3H mice. These intermediates and glucose were isolated, and their concentration and specific activities were determined. For mammary tumor tissue *versus* liver, the *in vivo* incorporation of these intermediates indicated a decrease in oxidative metabolism *via* the Krebs cycle but no observable differences in glycolytic metabolism.

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Studies on Therapy of Staphylococcal Infections in Monkeys. I. Comparison of Cloxacillin, Triacetyloleandomycin and Erythromycin.* (32304)

SAMUEL SASLAW AND HAROLD N. CARLISLE
Division of Infectious Diseases, Department of Medicine, Ohio State University College of Medicine, Columbus

Appropriate controlled studies for the comparison of antistaphylococcal chemotherapeutic agents in serious infections in humans have been difficult particularly because untreated controls can not and should not be employed. Various problems in the evaluation of therapy in staphylococcal infections have been reviewed recently(1). Previous studies from this laboratory(2) demonstrated that the rhesus monkey was an excellent experimental model for systemic staphylococcal infections. Since the experimental infections simulated those observed in natural infections in man, studies on therapy in monkeys could contribute to a better comparison of the efficacy of various antibiotics *in vivo*. To our knowledge, no studies on therapy of staphylococcal infections in subhuman primates have been described. In this report 2 bacteriostatic macrolide antibiotics are compared with a bactericidal semisynthetic penicillin.

Materials and methods. Thirty-one young adult monkeys *(Macaca mulatta)* weighing 2.8-4.5 kg were employed. Base-line observation included physical examinations, hematologic and bacteriologic studies for 2 weeks prior to challenge with a penicillin-resistant *Staphylococcus aureus,* phage type 80/81; tube dilution sensitivity tests(3) showed that the minimal inhibitory concentrations of cloxacillin, oleandomycin base and erythromycin were 0.25, 1.0 and 0.25 μg/ml, respectively, and bactericidal concentrations were 1.0, 31.3 and 15.6 μg/ml, respectively. Other characteristics of the staphylococcus and technics used in its preparation and intravenous inoculation have been described(2). Therapy with cloxacillin (Tegopen, Bristol,

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