hamsters to recover viruses or virus-like oncogenic agents from human malignant tissues. Altogether, 84 preparations from clinical specimens were tested. The human specimens were from leukemias and solid tumors and were tested as crude, filtered or fluorocarbon-treated extracts, as fluids from cell cultures of human malignant tissues, or as fluids from grivet monkey kidney cultures inoculated with clinical materials. There was a considerable incidence of spontaneous neoplasia, especially mammary carcinoma, among the mice but a very infrequent occurrence of malignancy in hamsters. Prevention of nursing among the mother mice did not increase the incidence of mammary carcinoma. Sex segregation of mice with retention of a virginal status appeared, in a small study, to diminish tumor incidence among females considerably below that of freely breeding animals. There was no statistically significant difference in tumor incidence among the experimental animals inoculated with specimens derived from human malignant tissues compared with the controls except for a few cases of leukemia which occurred in the experimental group and in a slightly more frequent occurrence of hepatoma and pulmonary adenocarcinoma among these animals. The need for adequate numbers of animals, for proper control procedures, and for extensive histologic examination of all abnormal animal tissues in studies of the role of transmissible agents in human malignancy was emphasized. The negative findings in these investigations were not interpreted as positive evidence for a non-viral etiology in malignancies of man.

E. A. Ridley, W. Raupp and T. Beddow contributed significantly to these studies. The specimens from human cases of malignancy were kindly supplied by Dr. Irving Wolman, Children's Hospital of Philadelphia, by Dr. H. T. Tamaki of Montgomery Hospital, Norristown, Pa., and by Dr. J. Gershon-Cohen of Albert Einstein Medical Center, Philadelphia. Collection of specimens by Dr. Wolman was supported in part by grant from U. S. Public Health Service.

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Catabolism of Glucose and Gluconate in Intact Rats. (27713)

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The mechanism for the catabolism of hexose in intact rats has been previously examined in several laboratories. Bloom *et al.*(1) concluded that the Embden-Meyerhof-Parnas (EMP) pathway is the principal, if not exclusive, mechanism for glucose utilization since

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no marked difference in $C^{14}O_2$ production was noted from rats injected with either glucose-1-C^{14} or glucose-6-C^{14}. Spiro and Ball(2), on the other hand, reported that C-1 was preferentially converted to CO_2 over C-6 of glucose. The findings were interpreted as indicating that the injected glucose was being metabolized by both the phosphogluconate and the glycolytic pathway. It has also been shown that the glucuronic acid cycle pathway, which involves the preferential conversion of C-6 of glucose to CO_2, is operative in intact rats(3).

To elucidate further the nature of glucose pathways in intact rats, several series of radiorespirometric experiments(4) have been carried out in which the utilization of C^{14} specifically labeled glucose or gluconate has been followed with respect to the time course for production of respiratory $C^{14}O_2$.

Materials and methods. Glucose-1-C^{14}, glucose-2-C^{14} and glucose-6-C^{14} were obtained from the National Bureau of Standards through the kind cooperation of Dr. H. S. Isbell. Glucose-U-C^{14} was obtained from Tracerlab Inc. Glucose-3(4)-C^{14} was prepared in this laboratory from liver glycogen, isolated from rats injected with sodium bicarbonate-C^{14}, according to the method of Wood, Lifson and Lorber(5). C^{14} specifically labeled gluconates were prepared from the correspondingly labeled glucose according to the method of Moore and Link(6). The purity of each of the labeled compounds was examined by means of paper chromatography and autoradiography.

Male albino rats (Sprague-Dawley, Northwest Rodent Farms, Pullman, Wash.) weighing 360-400 g were fasted for 48 hours prior to experiment. In the glucose experiments, C^{14} labeled glucose, approximately 2 μc in radioactivity and 1 to 2 mg in weight, is mixed with a prescribed amount of unlabeled glucose and dissolved in water to yield a solution 50% in concentration (W/V). In the gluconate experiments, each of the labeled gluconates, approximately 2 μc in radioactivity and 2 to 5 mg in weight, was mixed with 150 mg of unlabeled gluconolactone, 1500 mg of unlabeled glucose and 3 ml of distilled water. The solution of each of the labeled substrates was administered into the rat *per os*.

Following administration of the labeled substrate, the rat was placed in a respiratory chamber, 8 l in capacity. A stream of CO_2-free air was directed into the chamber at the rate of 200 ml per minute to sweep the chamber atmosphere continuously into a CO_2 absorption trap equipped with a sintered glass sparger. Twenty ml of a 3 N sodium hydroxide solution were used as the trapping reagent which was replaced every hour. Respiratory CO_2 trapped therein was recovered from the trap solution as barium carbonate by addition of a 1 N NH_4Cl-$BaCl_2$ solution. The $BaC^{14}O_3$ samples were mounted on aluminum planchets by means of the centrifugation technic and counted with a gas-flow Geiger Muller counter equipped with a thin mylar window (0.5 mg/cm^2). An adequate duration was allowed for the counting process so that the relative standard deviations of the counting data were always less than 2%. Counting data were corrected for background and self absorption in the conventional manner. Each of the radiorespirometric experiments was terminated when the specific activity of the respiratory $C^{14}O_2$ declined to a relatively low magnitude following the initial rise.

Results and discussion. To determine the optimal substrate level for the radiorespirometric experiments, rats were fed respectively 500, 1500 and 2500 mg of unlabeled glucose each containing 2 μc of glucose-U-C^{14}. The time course for the change in specific activity of $C^{14}O_2$ respired by these rats is given in Fig. 1. The data represent the average of results obtained in 3 identical experiments. As expected, the peak specific activities of $C^{14}O_2$ for different glucose levels appeared sooner with smaller amounts of glucose administered. It is also noted that relative specific activities of respiratory CO_2 samples are not proportional to relative specific activities of the original labeled glucose suggesting that a sizable pool of carbonaceous compounds exists in the rat, which is in equilibrium with the intermediates of glucose catabolism. This is analogous to the situation observed with tomato fruit(7).

In the earlier phase of the experiment, the

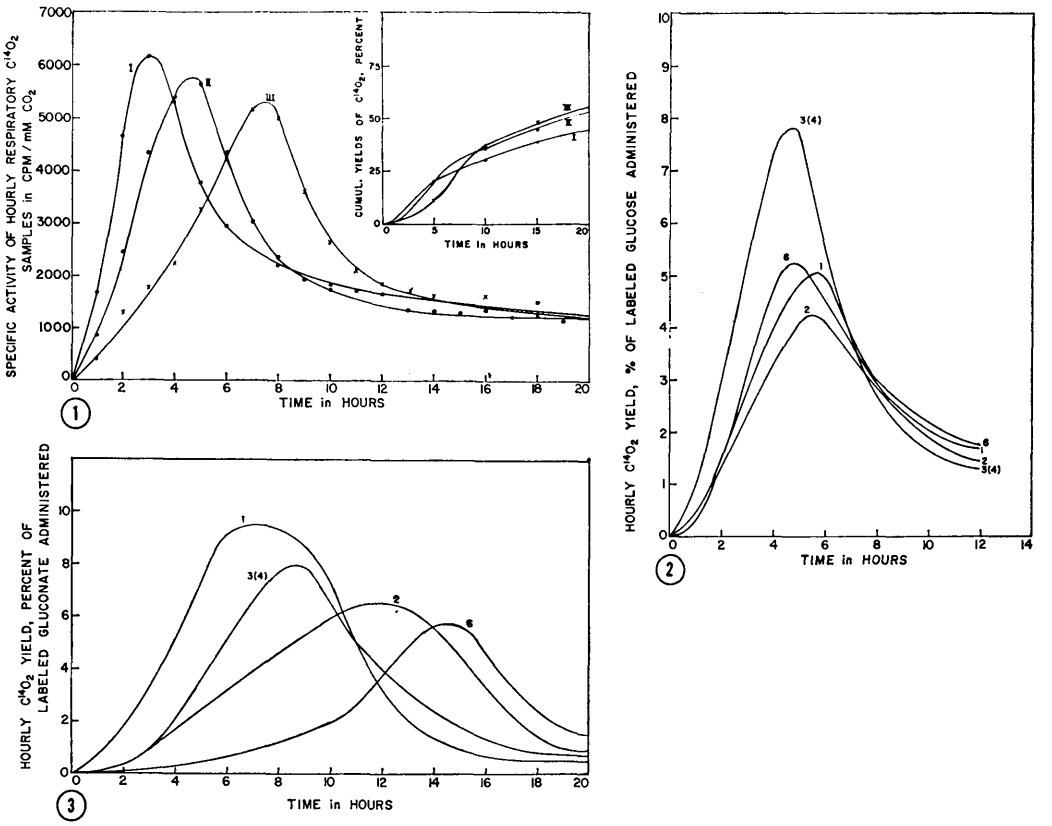

FIG. 1. Interval specific activities of respired CO_2 from rats metabolizing glucose-U-C^{14}. I = 2 μc of glucose-U-C^{14} in 500 mg of unlabeled glucose. II = 2 μc of glucose-U-C^{14} in 1500 mg of unlabeled glucose. III = 2 μc of glucose-U-C^{14} in 2500 mg of unlabeled glucose.

FIG. 2. Radiorespirometric pattern for rat metabolizing C^{14} specifically labeled glucose substrates. Numerals associated with each curve refer to position of carbon atoms of glucose labeled with C^{14}.

FIG. 3. Radiorespirometric pattern for rat metabolizing C^{14} specifically labeled gluconate substrates. Numerals associated with each curve refer to position of carbon atoms of gluconate labeled with C^{14}.

cumulative recoveries of substrate activity in respiratory CO_2 follow an anticipated order with respect to glucose levels. It was surprising, however, to find that the order was completely reversed at the end of the experiment, indicating that conversion of glucose carbon atoms to CO_2 is more extensive with increasing levels of the administered glucose. To evaluate the significance of this finding requires further experimentation, particularly since the levels of substrate involved in this experiment may have been considerably below the food intake capacity of the fasted rats.

Analysis of the data collected in the glucose-U-C^{14} experiment with respect to the reproducibility of the radiorespirometric data and optimal duration of the experiment led to the conclusion that subsequent experiments should be carried out with 1500 mg of glucose for each rat. In Fig. 2 and Table I are given the radiorespirometric data for utilization of glucose-1, -2, -3(4) or -6-C^{14} by intact rats. The data represents the average of 3 identical experiments, the variation among replica experiments being approximately 3%.

The prominent role played by the EMP pathway, in conjunction with pyruvate decarboxylation, in rat catabolism is clearly indicated by the prompt and extensive recovery of C-3(4) of glucose in respiratory CO_2. Of more interest is the finding that both C-6 and C-1 of glucose were converted to respiratory

TABLE I. Cumulative $C^{14}O_2$ Yields from Rats Metabolizing C^{14} Specifically Labeled Glucose Substrates.

Time, hr	$C^{14}O_2$ yields, % of total substrate administered: Glucose -1-C^{14}	Glucose -2-C^{14}	Glucose -3(4)-C^{14}	Glucose -6-C^{14}
2	2	2	4	2
4	9	7	17	10
6	18	15	30	20
8	25	22	37	27
10	30	26	40	31
12	33	29	43	35

CO_2 more extensively than that of C-2. This fact suggests strongly that pathways other than the EMP route are also operative for utilization of glucose by rats since preferential oxidation of C-2 and C-5 of glucose over that of C-1 and C-6 is anticipated in the classical EMP-Kreb's cycle sequence. The preferential conversion of C-1 of glucose to CO_2, in comparison to C-2, can probably be accounted for by the operation of the phosphogluconate decarboxylation pathway demonstrated in rat previously by Spiro and Ball(2). The preferential conversion of C-6 of glucose to CO_2 over that of C-2 presumably reflects the operation of the glucuronic acid cycle pathway reported by Eisenberg and coworkers(3).

The operation of the phosphogluconate decarboxylation pathway, in intact rats, is further confirmed by the average radiorespirometric data for utilization of C^{14} specifically labeled gluconate samples given in Fig. 3 and Table II, observed in 3 identical experiments. Under the assumption that the administered gluconate is in perfect equilibrium with the gluconate derived from glucose in the rat, much of the mechanism involved in utilization of glucose *via* the phosphogluconate decarboxylation pathway can be concluded by an analysis of these data. The prompt appearance of C-1 of gluconate in the respiratory CO_2 left little doubt that the administered gluconate was readily utilized by rat *via* a pathway involving a C_1-C_5 cleavage. Previous work by other groups with mammalian tissues, reviewed by Katz(8), suggests that the C_1-C_5 cleavage is in the nature of the phosphogluconate decarboxylation pathway giving rise to pentose phosphate. The fate of the pentose phosphate so formed in rat catabolism is of much interest. The conversion of pentose phosphate to fructose-6-phosphate (F-6-P) and then to glucose-6-phosphate (G-6-P) *via* the action of transketolase, transaldolase and phosphoglucoisomerase constitutes the pentose cycle pathway. The participation of this cyclic pathway in hexose catabolism has been recently examined by Katz and Wood(9), with respect to various theoretical considerations, although experimental evaluation of the role of this pathway has not yet been reported.

TABLE II. Cumulative $C^{14}O_2$ Yields from Rats Metabolizing C^{14} Specifically Labeled Gluconate Substrates.

Time, hr	$C^{14}O_2$ yields, % of total substrate administered: Gluconate -1-C^{14}	Gluconate -2-C^{14}	Gluconate -3(4)-C^{14}	Gluconate -6-C^{14}
4	11	3	3	1
8	46	19	26	4
12	70	43	50	13
16	75	62	59	33
20	77	68	62	42

In the present work, the rates of $C^{14}O_2$ production from the labeled carbon atoms of gluconate follow the order of C-1 > C-3(4) > C-2 > C-6. These results suggest that much of the pentose phosphate formed in the phosphogluconate decarboxylation was catabolized further *via* the sequence shown in Fig. 4. The conclusion thus also implies that only a minor portion of the fructose-6-phosphate was catabolized *via* the pentose cycle pathway.

The data presented here are not sufficient for estimation of the relative participation of individual pathways, since the existing methods in this regard are concerned with the concurrent operation of only 2 catabolic routes. Nevertheless, examination of the cumulative $C^{14}O_2$ data in the glucose experiment (Table I) led one to believe that the EMP pathway is probably the most important mechanism in utilization of glucose by rats. Thus, at the end of the experiments, 43% of the administered radioactivity in C-3(4) of glucose has appeared in CO_2 with the EMP-pyruvate decarboxylation sequence being, presumably, the principal mechanism. The other 2 catabolic routes, *i.e.*, phosphogluconate decarboxylation and glucuronate de-

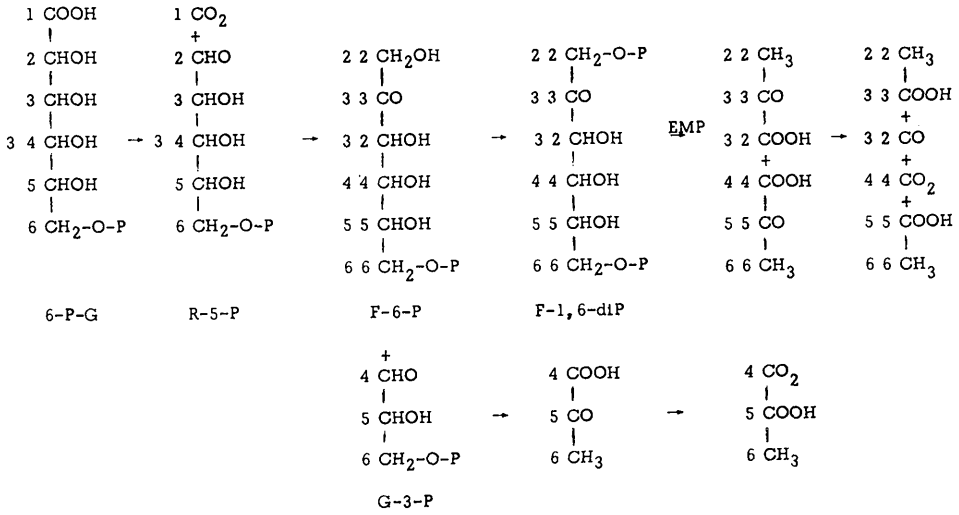

FIG. 4. Catabolic mechanism for utilization of gluconate by rat. 6-P-G = 6-phosphogluconate, R-5-P = ribose-5-phosphate, F-6-P = fructose-6-phosphate, F-1, 6-diP = fructose-1, 6-diphosphate, G-3-P = glyceraldehyde-3-phosphate.

carboxylation, therefore, appear to be minor pathways which may account for utilization of less than half of the administered glucose.

Summary. When glucose is administered to intact rats *per os*, it is catabolized mainly by way of the EMP pathway. Some of the administered glucose was also routed into the phosphogluconate decarboxylation and glucuronate decarboxylation pathways. Gluconate can be readily utilized by intact rats *via* the phosphogluconate decarboxylation pathway. The pentose phosphate so formed is believed to be converted to F-6-P which in turn is further catabolized primarily by way of the EMP route.

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Effect of Pyrogens on Urine Tonicity and Renal Papillary Sodium Concentration in the Rat.* (27714)

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Large intravenous doses of a pyrogen to the dog results in a diuresis which is associated with an increased solute excretion, a fall in urine osmolality, and is uniformly associated with a rise in total renal blood flow(1,2). In the dog, the fall in urine osmolality (and refractoriness to ADH) has been related to a

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