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Uptake of ^{67}Ga in the Regenerating Rat Liver and Its Relationship to Lysosomal Enzyme Activity[1]

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SUMMARY

The uptake of ^{67}Ga citrate has been studied in the regenerating rat liver over a period up to 72 hr after partial hepatectomy. The concentration of ^{67}Ga was found to be maximal (four times that of controls) 42 hr after hepatectomy. This was shown to be related to lysosomal enzyme activity rather than to specific phases of the cell cycle, there being a highly significant correlation ($p < 0.001$) with aryl sulfatase activity.

In both regenerating and normal rat livers, it was shown that ^{67}Ga uptake is reduced when protein synthesis is inhibited by cycloheximide but is unaffected by inhibition of DNA synthesis by cytosine arabinoside.

INTRODUCTION

Since the original observation by Edwards and Hayes (12) that ^{67}Ga concentrated in the involved lymph nodes of a patient suffering from Hodgkin's disease, this nuclide has been shown to be useful for imaging a wide range of human tumors. However, the mechanisms responsible for this uptake are not yet understood. Inflammatory lesions (4, 18) show increased uptake of ^{67}Ga, and also high uptake is seen in lactating mammary glands (13). Both increased metabolic activity and high rates of cell proliferation have been suggested as possible factors influencing ^{67}Ga uptake (3).

The near-synchronous wave of cell proliferation that occurs in rat liver following removal of two-thirds of the organ permits the study of the uptake of ^{67}Ga by dividing cells during different phases of the cell cycle. This paper describes some studies of the dynamics of ^{67}Ga uptake in regenerating rat liver over the 1st 72 hr after partial hepatectomy. The effects of specific inhibitors of DNA and protein synthesis on ^{67}Ga uptake are also presented.

MATERIALS AND METHODS

The rats used were 3-month-old males of the highly inbred Marshall strain weighing 180 to 200 g. Three rats were used at each time interval.

^{67}Ga citrate, carrier-free, was supplied by Philips-Duphar, Petten, Holland, and was injected at a 3% citrate concentration. Concentrations of ^{67}Ga were determined by well-scintillation counting. [6-^{3}H]TdR,[3] specific activity, 23 Ci/mmole; [5-^{3}H]uridine, specific activity, 26 Ci/mmole; and L-[^{14}C]leucine, specific activity, 348 mCi/mmole, were supplied by the Radiochemical Centre, Amersham, England. The ^{3}H and ^{14}C in aqueous tissue extracts were measured in a Packard Tri-Carb liquid scintillation counter, using a scintillator mixture of toluene and Triton X-100 (70:30), containing PPO (7 g/liter) and POPOP (450 mg/liter).

ara-C (Cytarabine; Upjohn, Crawley, England) and CYH (Sigma Chemical Co., London, England) were dissolved in 0.9% NaCl solution at the required concentration just before use.

DNA was extracted from acidified liver homogenates by heating twice with 1 N $HClO_4$ at 70° for 20 min. DNA concentrations were determined by the diphenylamine method (6). RNA was extracted by incubation of the acid-precipitated homogenate with 0.3 N KOH at 37° for 1 hr, and RNA was determined by the orcinol method (28).

Blood Clearance and Liver Concentrations of ^{67}Ga in Normal Rats. The blood and liver concentrations of ^{67}Ga were measured over the 1st 24 hr after the i.v. injection of ^{67}Ga citrate (7 μCi). Blood samples (0.5 to 0.6 ml) were taken from the tail vein into heparinized syringes. The contribution of the blood-borne ^{67}Ga to the total apparent liver concentration was studied by tying off the left lateral lobe of the liver prior to perfusion of the remaining portion with 15 ml 0.9% NaCl solution by cannulation of the thoracic aorta. The concentrations of ^{67}Ga in a piece of the perfused median lobe and in a piece of the unperfused left lateral lobe were then measured. The difference represented the amount of ^{67}Ga present in the blood.

Uptake of ^{67}Ga in Regenerating Liver. Two-thirds of the liver of the rat were removed under ether anesthesia (19). Partial hepatectomies were performed between 1000 and 1400 hr to minimize the effects of any diurnal rhythms. At intervals thereafter, the rats were given i.v. injections of a mixture of ^{67}Ga citrate (10 μCi) and [^{3}H]TdR (100 μCi). After 2 hr, the rats were killed by cervical dislocation under ether anesthesia, the liver was perfused, and a piece of the remaining right lobe was removed and homogenized in 0.25 M sucrose. An aliquot of the homogenate was made 0.3 N

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[3] The abbreviations used are: TdR, thymidine; ara-C, cytosine arabinoside; CYH, cycloheximide.

with respect to KOH, counted for ^{67}Ga, and incubated at 37° for 24 hr. The KOH solution was acidified at 0° with 1 N $HClO_4$ to 0.2 N, washed with 0.2 N $HClO_4$, and DNA extracted. [^{3}H]TdR and DNA were determined in the extract.

Aryl sulfatase was measured in an aliquot of the homogenate, with nitrocatechol sulfate as substrate (11).

Inhibition of DNA and Protein Synthesis. The effects of ara-C and CYH on ^{67}Ga uptake were investigated both in the regenerating liver at 42 hr after partial hepatectomy, and in normal rats. The drugs were given by i.p. injection in 0.5 ml 0.9% NaCl solution; ara-C (250 mg/kg) 2 hr, and CYH (1 mg/kg) 30 min before injection of ^{67}Ga citrate (8 μCi). Either [^{3}H]TdR (30 μCi) or [^{3}H]uridine (20 μCi) was given in a mixture with the ^{67}Ga citrate. Protein synthesis was determined by the uptake of L-[^{14}C]leucine (2 μCi) injected i.p. 20 min before killing.

The liver homogenate was acidified and washed twice with 0.2 N $HClO_4$. Both RNA and DNA were extracted as described above, and the acid-insoluble protein residue was dissolved in 1 N NaOH. Protein was determined by the method of Lowry *et al.* (23).

RESULTS

Blood Clearance, and the Effect of Perfusion on ^{67}Ga Concentration in Liver. After injection of ^{67}Ga citrate i.v., there is first a rapid clearance followed by a slow elimination of the nuclide from the circulation, as shown in Chart 1*C*. These 2 components are characterized by half-lives of 45 min and 10.2 hr, respectively. During this period there is an accumulation of ^{67}Ga in the liver of up to 2% dose per g at 24 hr.

As seen in Chart 1, at 2 hr after injection, the blood concentration (*C*) is much higher than that of the liver (*B*), and the effect of perfusion is shown in the *upper part* of Chart 1, where it is seen that, at this time, 30% of the apparent concentration in the liver is due to the blood. Hence, in the following experiments in which rats were killed 2 hr after injection, perfusion of the liver was routinely performed.

Uptake of ^{67}Ga in the Regenerating Liver. As is well known, after partial hepatectomy, the remaining liver is stimulated to rapid growth in a synchronous manner, with DNA synthesis beginning at 18 to 20 hr after hepatectomy (17). This is shown in Chart 2, in which the rate of DNA synthesis is plotted against time. A 2nd wave of DNA synthesis occurs at 42 hr, although the peak is less pronounced, due to loss of synchrony.

The ^{67}Ga concentration shown in Chart 2 is characterized by a 4-fold increase compared with normal liver 42 hr after hepatectomy, and is at a minimum during the S phase of the cell cycle at 20 to 24 hr. There is also a 3-fold increase in concentration 3 hr after hepatectomy.

Relationship of ^{67}Ga Uptake to Aryl Sulfatase Activity in Regenerating Liver. Because of the variations of production and activity of many enzymes during the cell cycle (26), the activity of the enzyme, aryl sulfatase, in the regenerating liver was investigated. This enzyme, which is located within the lysosomes, was used as an indicator of lysosomal

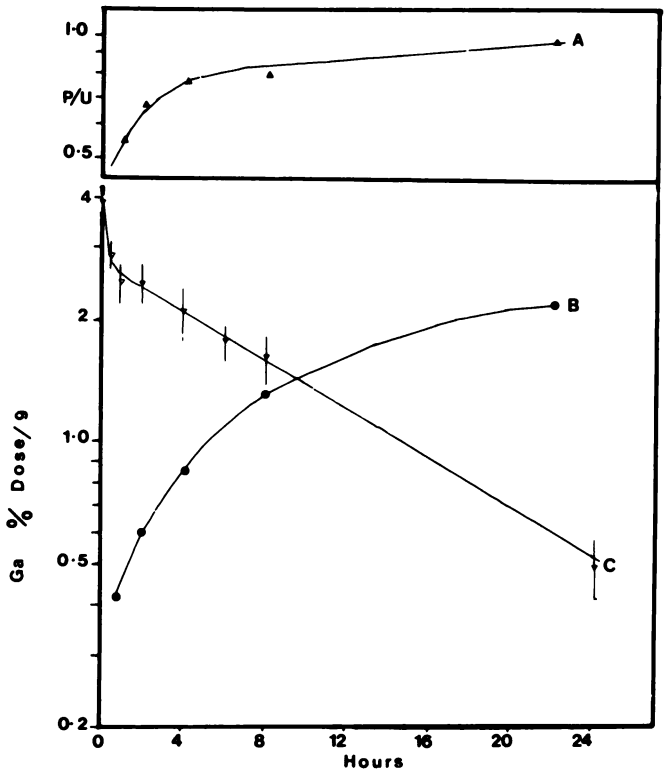

Chart 1. Variation of ^{67}Ga concentrations in blood and liver with time after injection. *C*, blood samples were taken from the tail vein; *vertical bars*, S.D. *B*, concentration in the perfused liver (see "Materials and Methods" for details); *A*, ratio of ^{67}Ga in perfused to that in unperfused livers.

enzyme activity. The results are shown in the *upper part* of Chart 2. It can be seen that there is an elevated level of enzyme activity at 42 hr, and that the enzyme level is closely followed by the ^{67}Ga concentration.

There is a highly significant correlation (correlation coefficient, 0.83; $p < 0.001$) between ^{67}Ga uptake and aryl sulfatase activity, as seen in Chart 3, in which results from individual animals are shown.

Effect of Inhibitors of DNA and Protein Synthesis on ^{67}Ga Uptake. The effects of ara-C, a specific inhibitor of DNA synthesis (15), and of CYH, a potent inhibitor of protein synthesis (31, 32), were investigated both in the regenerating liver 42 hr after partial hepatectomy and in the normal rat liver (Chart 4). CYH effects a marked reduction of protein synthesis in the regenerating liver, and the ^{67}Ga uptake is also reduced to one-half of the control value. DNA synthesis is almost completely inhibited by ara-C, but this agent has little effect on ^{67}Ga concentration.

In normal rats, in which protein and DNA rates of synthesis are lower, these drugs had no significant effects on the ^{67}Ga uptake.

DISCUSSION

The isotope ^{67}Ga is widely used as a scanning agent which concentrates in certain tumors, especially in Hodgkin's disease (21), malignant lymphoma (16), and lung carcinoma (9). It has also been shown to concentrate in inflammatory

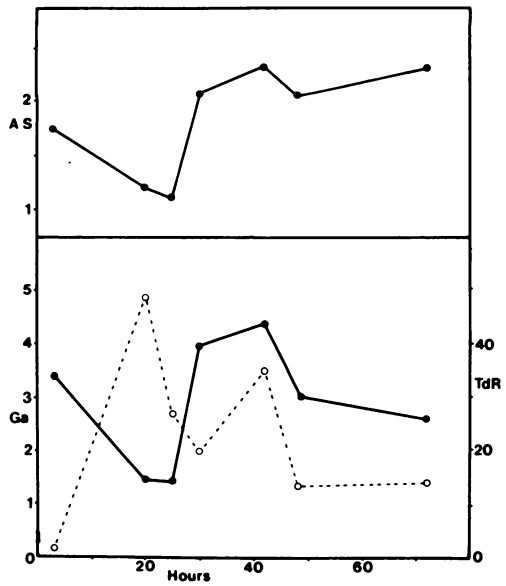

Chart 2. Concentration of ^{67}Ga in the regenerating rat liver at different times after partial hepatectomy (——). Concentration (% injected dose per g) expressed as ratio to control animals. - - - -, rate of DNA synthesis (incorporated [^{3}H]TdR, specific activity, cps/mg DNA) as a ratio of controls. Animals were killed 2 hr after injection of ^{67}Ga and [^{3}H]TdR. At the *top* of the chart, the aryl sulfatase activity (*A S*) is shown as a ratio to the control (expressed as μmoles nitrocatechol sulfate hydrolyzed at 37° in 2 hr).

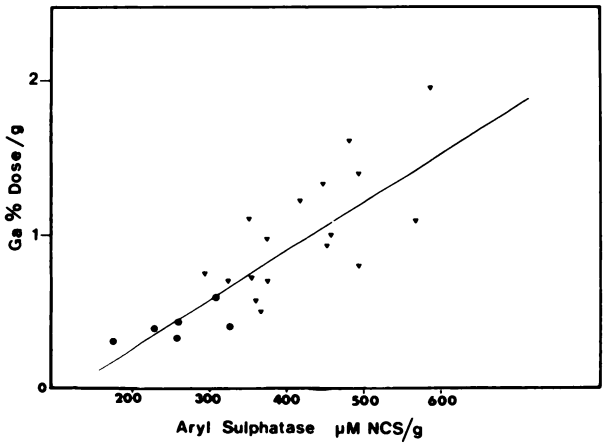

Chart 3. Concentration of ^{67}Ga in regenerating rat liver and aryl sulfatase activity (μmole nitrocatechol sulfate hydrolyzed per g per 2 hr at 37°). The regression line was calculated using a computer program (correlation coefficient, 0.83); ●, unoperated controls; ▼, regenerating after partial hepatectomy. *NCS*, nitrocatechol sulfate.

lesions (14, 22). It has been suggested that this may be due to proliferating, metabolically active cells. This hypothesis has received support from a study of an ascitic tumor in mice, the cells of which showed increased ^{67}Ga concentration in the recurrent growth phase compared with those in the plateau phase. It was also shown that ^{67}Ga concentration is enhanced in hyperactive bone marrow (3).

The regenerating rat liver offers a system in which ^{67}Ga uptake can be studied in its relationship to synchronous cellular proliferation. In order to study the kinetics of such a system, the time between injection and killing should be as short as possible. An interval of 2 hr was chosen to give sufficient concentration of ^{67}Ga (1% dose/g). At this time, the concentration of ^{67}Ga in the blood is 2.5% dose/g, and the ratio of ^{67}Ga in perfused to unperfused liver is 0.67. These results demonstrate the necessity of perfusion before meaningful data can be obtained.

The results in Chart 2 show clearly that the rate of ^{67}Ga incorporation into the regenerating liver varies considerably with time. At 3 hr after partial hepatectomy, the ^{67}Ga concentration is about 3 times that found in normal liver but, thereafter, the concentration falls to a minimum at the peak of DNA synthesis before rising to reach a maximum of 4 times the normal liver value at 42 hr after operation. This is contrary to the observation of Orii (27) who found no increase in ^{67}Ga concentration at 42 hr after partial hepatectomy. Recently, Hill and Wagner (20) have argued that ^{67}Ga uptake is not a function of the rate of cellular proliferation *per se*, since they observed no increase in concentration in the regenerating liver during the period 16 to 24 hr after surgery. However, this interval coincides with the period of DNA synthesis, and our results show that ^{67}Ga uptake is minimal during this phase of the cell cycle, although the concentration in regenerating liver is still 1.4 times greater than that in normal liver.

It can be seen from Chart 2 that the variation in ^{67}Ga uptake mirrors the level of aryl sulfatase activity found in the regenerating liver. When ^{67}Ga concentrations in the livers of individual animals are plotted against their aryl sulfatase activities, there is a highly significant correlation ($p < 0.001$) (Chart 3). The reason for the increase in

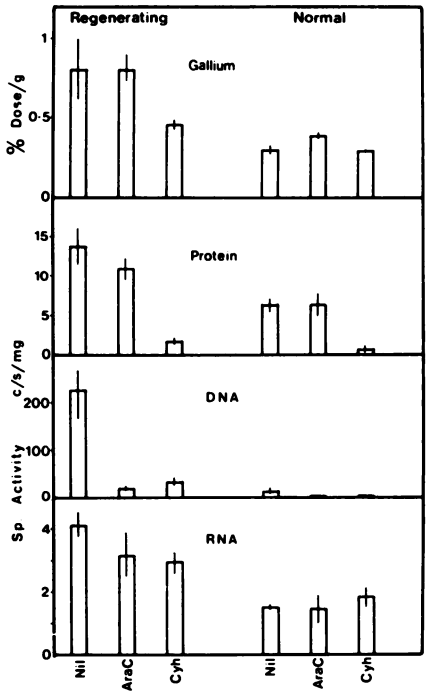

Chart 4. Effects of ara-C and CYH on ^{67}Ga uptake, and rates of protein, DNA, and RNA synthesis both in the regenerating rat liver 42 hr after partial hepatectomy, and in normal livers. Controls, without drugs, are shown as *Nil*. *Vertical bars*, S.D. for 3 animals in each group; *c/s/mg*, cps/mg; *Sp.*, specific.

lysosomal enzyme content after hepatectomy is not known. Similar increases in aryl sulfatase and other lysosomal enzymes were found by Dianzani (10) in rats 48 hr after they had been given carbon tetrachloride, and this could indicate a general response to injury by the remaining tissue cells. This could be related to the appearance of large vacuoles containing acid phosphatase, which have been reported by many observers to occur after partial hepatectomy and other hepatic injuries (29). Alternatively, it has been shown that degenerating cells lose lysosomal enzymes less rapidly than other normal tissue constituents, so that there is an apparent increase in activity as the tissue loses weight or protein nitrogen (8). This is consistent with the uptake of ^{67}Ga in inflammatory tissues and in neoplasms, where there is a larger proportion of necrotic cells than in normal tissue. This is confirmed by our findings (P. A. G. Hammersley and D. M. Taylor, unpublished data) that the concentration of ^{67}Ga in animal tumors is increased at 24 to 48 hr after X-irradiation or chemotherapy, and is accompanied by a dramatic increase in aryl sulfatase activity.

The results reported here suggest that ^{67}Ga uptake is related to the lysosomal content of regenerating liver cells, as represented by aryl sulfatase, rather than to the rate of cellular proliferation, as such. It has been well established that ^{67}Ga concentrates in the lysosomal fraction of cells in liver tissue (2) and in a variety of animal (5, 30) and human (unpublished results) tumors. The results of several experimental (14) and clinical (1, 25) investigations may be explained on the basis of increased lysosomal content being responsible for the observed ^{67}Ga accumulation.

The results of the experiments using CYH and ara-C suggest that ^{67}Ga uptake is related to the rate of protein synthesis and is independent of DNA synthesis. There is no obvious relationship to the rate of RNA synthesis. These results are consistent with the variation of ^{67}Ga with time after hepatectomy (Chart 2). After DNA synthesis and mitosis, there is renewed protein (including lysosomal enzyme) synthesis. Also, the enhanced concentration of ^{67}Ga 3 hr after hepatectomy occurs in a period of rapid protein (24) and ribosome (7) synthesis. This relationship with protein synthesis is also supported by the fact that the concentration of ^{67}Ga in normal tissue is greatest in those organs in which there is a relatively large amount of protein synthesis, including the liver, kidney, gastrointestinal epithelial tissue, and the macrophage system.

The nature of the relationship between ^{67}Ga uptake and aryl sulfatase activity is not yet clearly understood. The correlation between aryl sulfatase and ^{67}Ga concentration may not extend to lysosomal enzymes in general. Further work is continuing in order that we may examine the nature and extent of this hypothesis in other proliferating systems and in animal tumors.

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