which produced our own atmosphere (*4*). I also adopt this assumption. If we then attempt to assess the total terrestrial volatile inventory, allowing for biological and hydrological effects, the resulting abundance ratios for the various gaseous constituents should be applicable in a general way to the other two planets. This estimate cannot be made with great precision, but we are only interested in orders of magnitude and the pioneering work by Rubey (*5*) is well suited for this purpose.

The constituents of greatest significance are carbon dioxide, argon, and neon, which are present in the earth's volatile inventory in the approximate ratio $3 \times 10^6 : 1.1 \times 10^3 : 2.2$. Nitrogen could be useful, but it is susceptible to chemical deposition and escape, while neon and argon, once in the atmosphere, will remain there. This helpful property is retained even with a cold trap temperature of 145°K, the present estimate for the lowest temperature at the polar caps on Mars. In other words, when large amounts of H_2O and CO_2 are deposited at the martian poles during putative ice ages, the neon and argon will become relatively more abundant in the atmosphere.

It is well established that more than 99 percent of the argon in our own atmosphere is ^{40}Ar produced by the decay of ^{40}K in rocks and subsequently released in the various outgassing processes that have produced the other constituents (*6*). In contrast, terrestrial neon and ^{36}Ar appear to be primordial in the sense that they have been released from the materials that originally accreted to form the earth. Whatever mechanism was responsible for the fractionation of the noble gases observed in the earth's atmosphere seems to have produced identical results in the planetary component of trapped meteoritic gases (*7*). We thus expect $^{20}Ne : {}^{32}Ne : {}^{36}Ar$ to be similar on Mars and the earth, since Mars presumably accreted from material that had a similar history in this respect.

Using this information on terrestrial rare gas abundances, I calculate that 1 bar of CO_2 trapped at the martian poles implies that the present atmosphere (surface pressure $\sim$ 5.5 mbar) should contain approximately 0.33 mbar of ^{40}Ar. On the other hand, if most of the outgassed CO_2 is still in the gas phase, so the surface pressure is now as high as it ever was, we should expect only 2×10^{-3} mbar of ^{40}Ar to be present, or 0.04 percent of the present atmosphere. This range in argon abundance is encompassed by the dynamic range of the mass spectrometer presently under development for the Viking mission of the National Aeronautics and Space Administration in 1976 (*8*). We have every reason to expect that our colleagues in the Soviet Union have developed similar instrumentation, which means that the required measurements may be made after the soft landing next February of Soviet spacecraft presently en route to the planet (*9*).

In other words, a simple, terrestrial analog model for the origin of the martian atmosphere requires the release of enough ^{40}Ar to serve as a sensitive tracer for the total amount of CO_2 outgassed by the planet and thus provides an index by which to judge the credibility of cyclic models for martian climatology. In fact, this test may be used to determine whether the atmosphere was ever more massive than it is at present, even if this stage occurred only once in the planet's history. A check on the applicability of terrestrial analogy will be provided by a determination of the relative abundance of ^{40}Ar compared to ^{36}Ar, ^{20}Ne, and ^{22}Ne, for the reasons described above. For example, if the present atmosphere is actually a relic from a time of early, massive, accretional outgassing (*10*), we would expect an anomalously high concentration of neon and relatively little ^{40}Ar.

Some qualifications of this argument must be added. It is possible that the distribution of ^{40}K in the upper layers of Mars and the earth is not identical and hence the outgassing of ^{40}Ar and CO_2 may not have maintained the same ratio on Mars as on the earth. But this will be a small effect compared with the factor of 200 that separates the relative abundances of argon expected in the models under consideration. It must also be recognized that the argon-neon test cannot prove that polar reservoirs exist because an anomalously high ^{40}Ar concentration does not guarantee that the missing CO_2 is all trapped at the poles. Some may have formed carbonate rocks, and dissociation and escape cannot be completely ruled out (*11*). On the other hand, it is difficult to imagine the presence of a large reservoir of CO_2 without some anomaly in the argon and neon abundances. It should therefore be easier to dismiss cyclic theories than to prove their validity, but it will also be possible to provide a good test of whether or not the martian atmosphere was ever much more massive than it is today. These analyses will gain in acuity from whatever additional information can be obtained on the chemical and elemental composition of the crust and the escape processes that have operated in the upper atmosphere. Experiments that should produce information in both of these categories are included on the Soviet and American spacecraft.

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Galactosemia and Galactonolactone: Further Biochemical Observations

A rapid method of detecting a galactosemic fetus using only a few cells biopsied from amniotic fluid was recently proposed by Hill and Puck (*1*). In this procedure γ-D-galactonolactone, a presumed inhibitor of one of the alternate pathways for galactose utilization, was used. Incorporation of [1-^{3}H]galactose into trichloroacetic acid (TCA) precipitable material was monitored by radioautography (*1*). Approximately 23 silver grains per galactosemic cell were detected in comparison to over 400 grains per nongalactosemic cell when both were grown for 16 hours in the presence of 0.05*M* γ-D-galactonolactone and [1-^{3}H]galactose (*1*). This procedure allowed a small number of galactosemic cells to be more readily distinguished from nongalactosemic cells. Hill and Puck reported that without galactonolactone

more than 500 and 1000 silver grains were found per galactosemic and nongalactosemic cell, respectively. They suggested that 0.05*M* γ-D-galactonolactone inhibited in vitro a major alternate pathway which does not require galactose-1-phosphate uridyltransferase. This leaves galactosemic cells with one remaining minor metabolic route leading to TCA insoluble material, as compared to one minor and one major metabolic route for the nongalactosemic cells similarly treated with galactonolactone.

According to our data, there is a different explanation for the galactonolactone mediated differential inhibition of the utilization of galactose by nongalactosemic and galactosemic human fibroblasts under the conditions described by Hill and Puck (*1*). Solutions of 0.05*M* γ-L-galactonolactone and γ-D-galactonolactone in phosphate-buffered saline (*p*H 7.2) or in various tissue culture media initially at *p*H 7.4 became progressively acidic. The spontaneous degradation of galactonolactone to an acid has been observed by other investigators (*2*). Tissue culture media such as Eagle's minimal essential medium (MEM), F-12, and L-15 are not capable of buffering the amount of acid spontaneously formed from 0.05*M* γ-D- or γ-L-galactonolactone. There is no indication by Hill and Puck that the *p*H of the tissue culture media containing 0.05*M* galactonolactone was monitored during the incubation period. Under the conditions described by Hill and Puck, the *p*H of the F-12 media containing 0.05*M* γ-D-galactonolactone after a 4- and 17-hour incubation period would have been approximately 6.2 and 4.5, respectively. We investigated the possibility that the reduction in *p*H of the tissue culture media could account for part or all of the diminished galactose utilization in nongalactosemic cells and nearly complete inhibition in galactosemic cells after a 16-hour incubation period with 0.05*M* γ-D-galactonolactone.

Nongalactosemic (galactose-1-phosphate uridyltransferase positive) human fibroblasts were provided by B. W. Uhlendorf, and galactose-1-phosphate uridyltransferase–deficient fibroblasts (GM52 and GM53) were purchased from the Institute for Medical Research (*3*). Cells were tested in L-15 media free of antibiotics and hexose (*4*), but supplemented with 10 percent fetal calf serum and buffered to the appropriate *p*H with phosphate. The cells were cultured at 37°C in Optilux tissue culture dishes (100 by 20 mm; Falcon Plastics, Oxnard, California), with 10 ml of media in each dish. The number of preconfluent growing cells per dish was counted before and after the treatment, and all data (Table 1) were corrected for the slight variation in cell number among replicate dishes after the treatment period.

Table 1. The effect of 0.05*M* γ-D-galactonolactone, γ-L-galactonolactone, and low *p*H on [1-^{14}C]galactose incorporation into TCA insoluble material in galactosemic and nongalactosemic cells in L-15 media. Cells were grown in Eagle's MEM and transferred to hexose-free L-15 media prior to treatment. The mean number of counts per minute of [1-^{14}C]galactose incorporated into TCA insoluble material for approximately 2.05 × 10^5 preconfluent growing nongalactosemic cells per petri dish in the 17-hour incubation period was 56,831 count/min, which is equivalent to 100 percent in this table. The numbers in parentheses represent the number of independent determinations.

Treatment	Initial *p*H	*p*H after 17-hour treatment	Incorporation of [1-^{14}C]galactose (%)
Nongalactosemic cells			
None	7.2	7.5	100 (6)
Low *p*H	6.0	6.1	65.5 (3)
Low *p*H	5.5	5.6	22.3 (2)
0.05*M* γ-L-galactonolactone	7.2	4.6	41.6 (2)
0.05*M* γ-D-galactonolactone	7.2	4.7	28.7 (4)
Galactosemic cells			
None	7.2	7.4	51.9 (6)
Low *p*H	6.1	6.2	19.9 (4)
Low *p*H	5.5	5.7	3.5 (2)
0.05*M* γ-L-galactonolactone	7.2	4.6	0.8 (2)
0.05*M* γ-D-galactonolactone	7.2	4.9	2.6 (5)

A 1.0*M* galactonolactone solution in buffered saline was brought to *p*H 7.0 with NaOH, and 0.5 ml was immediately added to 10 ml of L-15 media per petri dish. After 1 hour, 2.5 μc of [1-^{14}C]galactose (New England Nuclear, 52.9 mc/mmole) was added. After the 17-hour treatment period, the cells were washed three times in 1.0 ml of phosphate-buffered saline without Ca^{2+} or Mg^{2+} and removed by scraping with a rubber policeman. The dishes were washed twice in 1.0 ml of buffer. Cold 10 percent TCA was added to the cell suspension to give a final concentration of 5 percent TCA. The cells were maintained at 4°C for 10 minutes and then pipetted onto Gelman glass fiber filters, type A. The filters were washed three times with 5 percent TCA, dried, and the radioactivity was counted in a Beckman liquid scintillation spectrometer.

Both galactosemic and normal cells incorporated [1-^{14}C]galactose into TCA insoluble material. Galactosemic cells incorporated 51.9 percent of the nongalactosemic amount of TCA insoluble material from [1-^{14}C]galactose, which is consistent with previous observations (*1*, *5*). Galactonolactone reduced the [1-^{14}C]galactose incorporation into TCA insoluble material by galactosemic cells to approximately 2.6 percent (Table 1). These results are similar to those reported by Hill and Puck. They observed a reduction of approximately 2 to 4 percent or 23 ± 9 grains per galactosemic cell in comparison to more than 1000 grains for nongalactosemic cells.

After 16 hours, the *p*H of the media containing 0.05*M* galactonolactone was approximately 4.8 (Table 1). Adjusting the L-15 media to a *p*H of 5.5 with either NaH_2PO_4 or concentrated HCl also reduced the conversion of [1-^{14}C]-galactose into TCA insoluble material in galactosemic and nongalactosemic cells to levels which are similar to those found after treatment with 0.05*M* galactonolactone (Table 1). Given the conditions described by Hill and Puck the reduced *p*H due to the spontaneous degradation of commercially available galactonolactone (*6*) to an acid (*2*) may account for a substantial portion of the effect of galactonolactone on galactose metabolism in galactosemic and nongalactosemic cells.

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7. We thank M. F. Damuth of the NIH Media Unit and P. Friedman for assistance.

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Friedman, Yarkin, and Merril were kind enough to give us a preliminary report of their findings on galactonolactone inhibition. We have also been studying the effect of *p*H on galactosemic cells, and we have confirmed their observations. We now believe that the differential effect of D-galactono-γ-lactone on galactose incorporation in nongalactosemic and galactosemic cells is most likely due to its effect on *p*H rather than to its involvement in an alternate pathway for galactose metabolism.

We now report our experiments designed to achieve a satisfactory control of *p*H and to measure the effect of added lactone (*1*) on radioactive galactose incorporation. Several changes were made from our previous experimental protocol (*2*). (i) Cells (2×10^5) were incubated overnight in 1 ml of the growth medium (*2*), and scintillation vials instead of welled slides were used for quantitation. The scintillation vial system was comparable to the slide system, and to the system used by Friedman *et al.* (ii) The test medium (*2*) was changed by the addition of 0.075*M* MES [2-(*N*-morpholino)ethanesulfuric acid] buffer (*3*), and 4 μc of [1-^{14}C]galactose (New England Nuclear; specific activity 52.9 mc/mmole). One milliliter of this medium was used in each scintillation vial. (iii) The vials were incubated at 37°C in air. (iv) The incubation time was shortened to 6 hours, an interval long enough to permit sufficient incorporation into both types of cells [table 1 in (*2*)] in the absence of inhibitors. (v) The concentration of lactone was reduced from 0.05*M* as in (*2*) to 0.025*M*. We have ascertained that as little as 0.01*M* lactone will produce a significant differential effect on galactose incorporation in the scintillation vial system, when incubated for 4 hours in the absence of added buffer. The last two modifications should tend to reduce the effect of lactone in lowering the *p*H.

The *p*H was read immediately before the incubation period, and at the termination of each experiment. The cells were fixed with 10 percent formalin in 0.15*M* NaCl and washed (*2*). RPI Scintillator (Research Products International Corp.) in seven parts of toluene to three parts of Triton X-100 was added directly to the dried vials. The fixed cells were salvaged after counting, and the protein content was assayed (*4*) to be sure that no cell loss had occurred. The coefficient of variation for duplicate vials with respect to protein was 16.4 percent and for disintegrations per minute was 7.3 percent. The protein content from pair to pair of vials did not differ significantly, regardless of the treatment. The cell strains used were GM52 (the galactosemic line employed by Friedman *et al.*) and a nongalactosemic line (S120) from a foreskin biopsy (*5*).

Table 1. Percentage of incorporation of [1-^{14}C]galactose into material insoluble in trichloroacetic acid. Nongalactosemic and galactosemic cells were treated as described in the text. All samples were done in duplicate. The lactone concentration was 0.025*M*; 100 percent is equal to 3.0×10^5 disintegrations per minute of [1-^{14}C]galactose incorporated.

Treatment	Initial *p*H	*p*H after 6 hours	Incorporation (%)
	Nongalactosemic cells		
None	6.5	6.4	100
Lactone	6.5	6.2	101
None	5.5	5.0	28
Lactone	5.5	5.4	31
None	5.0	5.0	6
Lactone	5.0	4.7	6
	Galactosemic		
None	6.5	6.4	51
Lactone	6.5	6.1	49
None	5.5	5.1	12
Lactone	5.5	5.3	15
None	5.0	5.3	2
Lactone	5.0	4.8	2

The results of a typical experiment are summarized in Table 1, and confirm those of Friedman *et al.* reported above, except that the differential that we observed is less marked, probably because of the shorter incubation in our experiments. Control of *p*H was not perfect, but there were no significant differences in radioactive galactose incorporation at a given *p*H, whether lactone was present or not. When L-galactone-γ-lactone and D-glucono-δ-lactone were substituted for D-galactono-γ-lactone, no differences were observed which could not be ascribed to *p*H. Lactone at 0.05*M* was also tested, and no significant differences were observed.

We observed (*2*) about an 11-fold difference in grain counts between nongalactosemic and galactosemic cells in the presence of lactone after 4 hours of incubation. In the experiment depicted in Table 1, the greatest differential is only threefold. The explanation for this discrepancy may be that galactosemic cells incorporate galactose at a slower rate (*2*) and may also be more sensitive to low *p*H. Thus the time-dependent effect of lactone on *p*H may serve to amplify the difference in galactose incorporation between the two cell types.

Our previous observation (*2*) of a difference in metabolism of galactosemic and normal cells in vitro was thus correct, and the test we described is valid, although the mechanism which we proposed at that time was apparently erroneous; we believe that Friedman *et al.* have adequately explained our results. Our hypothesis for a general method to detect inborn errors of metabolism must therefore be tested in a different genetic disease with the use of other labeled biochemical intermediates.

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