tion of transfused stem cells in peripheral blood from normal and leukemoid rodents has been successful in radiation protection experiments (*13*). The number of circulating leukocytes required for successful radiation protection in rodents has been reported to be 100 times that necessary for radiation protection by bone marrow infusions. It is of interest that the dose of leukocytes transfused to our patients was in the range of 10^{11} cells, while the number of bone marrow cells transfused in the few cases in which "takes" have been demonstrated in man was 10^{9} cells.

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References

1. E. E. Morse, W. Bronson, P. P. Carbone, E. J. Freireich, *Clin. Res.* **9**, 332 (1961).
2. A. Kliman, L. A. Gaydos, L. R. Schroeder, E. J. Freireich, *Blood* **18**, 303 (1961).
3. J. Whang, E. Frei III, J. H. Tjio, P. P. Carbone, G. Brecher, *ibid.*, in press.
4. J. H. Tjio and J. Whang, *Stain Technol.* **37**, 17 (1962).
5. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. A. Hungerford, *Exptl. Cell. Res.* **20**, 613 (1960).
6. W. N. Valentine and W. S. Beck, *J. Lab. Clin. Med.* **38**, 38 (1951).
7. A. C. Peacock, G. Brecher, E. Highsmith, *Am. J. Clin. Pathol.* **39**, 80 (1958).
8. J. B. Block, P. P. Carbone, J. J. Oppenheim, E. Frei III, *Ann. Internal Med.*, in press.
9. R. H. Levin, E. Frei III, M. Landy, *Clin. Res.* **11**, 204 (1963).
10. M. J. De Vries, B. G. Grouch, L. M. Van Pritten, D. W. Van Bekkan, *J. Natl. Cancer Inst.* **27**, 67 (1961).
11. G. Mathé, J. Bernard, M. J. De Vries, L. Schwarzenberg, M. J. Larrieu, C. M Lalanne, J. L. Dutreix, T. Amil, J. Surmont, *Revue d'Hematol.* **15**, 115 (1960).
12. J. O. W. Bielby, I. S. Code, A. M. Jelliffe, D. M. Parben, J. W. Stewart, *Brit. Med. J.* **I**, 96 (1960).
13. J. W. Goodman, G. S. Hodgson, *Blood* **19**, 702 (1962).

18 September 1963

Galactose Metabolism by Rat Liver Tissue: Influence of Age

Abstract. *The metabolism of galactose by liver tissue from fetal, new-born, pre-adult, and adult rats was studied in vitro by determining the extent of conversion of galactose to CO_2 and the disappearance of the sugar from the incubation medium. Results indicate that the utilization of galactose by liver tissue decreases as the organism grows older. The explanation of the tendency of patients with congenital galactosemia to improve symptomatically with age on the basis of "maturation" of galactose metabolizing pathways in their livers does not appear to be consistent with these observations.*

Patients with congenital galactosemia have been shown to lack the enzyme, galactose-1-phosphate uridyl transferase (Gal-transferase) (*1*). The absence of this enzyme, which catalyzes the reaction,

galactose-1-phosphate (Gal-1-P) + uridinediphosphate-glucose (UDPG) → glucose-1-phosphate (G-1-P) + uridinediphosphate-galactose (UDPGal),

results in an inability to metabolize galactose, and this deficiency is associated with a syndrome in infants, characterized by malnutrition, vomiting, diarrhea, enlargement of the liver and spleen, jaundice, cataracts, and mental deficiency. It has been learned from clinical experience that as these patients grow older, especially after puberty, the ingestion of galactose may fail to elicit the symptoms typical of galactose intoxication. An explanation for this apparent amelioration of the clinical disease appeared when Isselbacher (*2*) reported the existence in mammalian tissue of an enzyme, uridine diphosphate galactose pyrophosphorylase (UDPGal-pyrophosphorylase), which could circumvent the enzymatic deficiency believed to be responsible for the galactosemic syndrome by catalyzing the reaction,

Gal-1-P + uridinetriphosphate → pyrophosphate + UDPGal

The activities of both enzymes, Gal-transferase and UDPGal-pyrophosphorylase, were shown to be five times greater (per milligram of tissue) in the livers of adult rats than in the livers of newborn rats (*2*). It seemed highly plausible that the galactosemic patient, though lacking the transferase, was capable of developing the pyrophosphorylase as he matured, thereby acquiring at least a partial ability to metabolize galactose.

Recent quantitative measurements of galactose oxidation in galactosemic subjects, in which galactose-1-C^{14} was used, revealed (*3*) that there exists among galactosemic patients a subgroup whose members develop, at some time during childhood, a substantial ability to metabolize galactose. Such acquisition of pathways for the metabolism of galactose was, however, observed in only a small proportion of the patients tested, and the majority of postpubertal galactosemic patients tested were unable to metabolize galactose, although they too exhibited the clinical amelioration of the syndrome described above. Therefore, it seemed worthwhile to evaluate the functional significance of the increased Gal-transferase and UDPGal-pyrophosphorylase activities observed in adult liver tissue, and to establish whether these heightened enzymatic activities were, in fact, reflected in augmentation of galactose metabolism by the tissue, as postulated (*2*). Experiments were performed to determine the ability of liver tissue from rats of various ages to consume galactose and to convert galactose to CO_2; consumption being taken as an index of the overall utilization of the sugar, and CO_2 production as an index of the extent of operation of the sequence of reactions participating in the catabolism of galactose to CO_2.

Figure 1 shows the striking extent to which galactose consumption by liver from newborn rats exceeded galactose consumption by adult liver tissue. Results from the first three experiments also showed that galactose consumption rises immediately after birth, and thereafter declines, so that liver tissue from 20-day-old rats yields intermediate values, and galactose consumption by adult rat liver is less than 50 percent of that by liver from newborn rats. In the experiments shown in Fig. 1, liver tissue was obtained from individual fetuses and newborns; other experiments, in which pooled liver tissue from entire litters was used, yielded results similar to those shown graphically.

Figure 2 shows that the rate of uptake by the younger tissue greatly exceeds that of the older tissue over an extended interval. (The linearity of uptake by the newborn tissue of one strain appears to fall off after 90 minutes, but this apparent effect is believed to be artifactual, and is attributed to the fact that the rapid rate of utilization by this tissue caused the concentration of substrate to fall below the level necessary to maintain saturation of the system and linear kinetics. The saturating con-

centration was shown to be approximately 20 mg per 100 ml in a series of similar incubations.) The failure of the curve to intercept the ordinate at zero is best explained by the distribution of sugar in the tissue water and the utilization taking place during the 3 minutes of tissue mincing and gas exchange before the timed incubation was started. The amount of tissue used in each incubation flask varied between 90 and 125 mg; an absolute proportionality between utilization of galactose and amount of tissue present was clearly demonstrated in experiments conducted for the purpose of excluding artifactual results which might be obtained if such proportionality were lacking.

Experiments were also conducted to show what effect (if any) the method

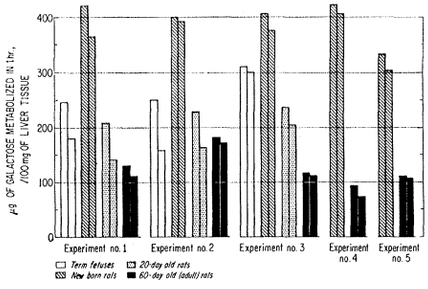

Fig. 1. Micrograms of galactose consumed in 1 hour per 100 mg of liver tissue from rats of varying ages. In the first four experiments, we measured galactose consumption by liver tissue from inbred rats of the Sprague-Dawley strain; in the 5th experiment we measured galactose consumption by liver from inbred rats of the Osborne-Mendel strain (*7*). Liver tissue was obtained from each animal just after killing by decapitation and was immediately suspended in 2 ml of Krebs-Ringer bicarbonate buffer to which 1 mg of D(+)galactose had been added. The tissue was then quickly chopped into tiny segments with fine scissors, and the air in the gas phase of the incubation flasks was replaced by a mixture of 5 percent CO_2 and 95 percent O_2 just prior to sealing and incubation at 37C° (with shaking, in a Dubnoff-type flask incubator). At the end of 60 minutes the incubation medium was assayed for the amount of galactose remaining by incubating 0.5 ml of a Somogyi-Nelson filtrate (at a dilution of 5 : 1) with galactose oxidase in the presence of peroxidase and benzidine (*8*) for 30 minutes, acidifying, measuring the absorption of ultraviolet light at 310 mμ (with a Beckman spectrophotometer), and comparing against a standard curve prepared from known galactose solutions. Each double set of bars depicts the actual number of micrograms of galactose consumed per 100 mg of tissue in simultaneous duplicate incubations.

of preparing the tissue could exert on the apparent rate of galactose utilization. In these experiments, it was found that the rate of galactose utilization per milligram of tissue did not differ significantly when tissue from the same animal was finely chopped (as described in the legend to Fig. 1), sliced (with a Stadie-Riggs microtome), or incubated in relatively large (up to 25 mg) intact fragments. The activity of tissue which had been subjected to rapid but vigorous mincing (by pressing and rubbing against a glass surface with the end of a spatula) was greatly reduced, while true homogenates of similar tissue possessed little or no activity. It seemed, therefore, that the results of this kind of incubation are reproducible within a fairly wide range of procedural variation, provided that the integrity of the hepatic architecture is largely preserved, and that the tissue fragments are small enough to allow the exchange of metabolites by diffusion. (Chopping, rather than slicing, was chosen for the experiments described in Figs. 1 and 2, because the fetal tissues lent themselves more readily to chopping.)

Data assessing the oxidation of galactose are shown in Table 1. Only a small fraction of the galactose taken up by tissue is oxidized all the way to free CO_2. However, in these experiments, oxidative metabolism of galactose showed the same relationship between newborn and adult tissue as was demonstrated for uptake; that is, the adult tissue oxidized about 50 percent less galactose to CO_2 than did the newborn tissue. Liver tissue from 14-day fetuses (7 days before term) was also studied, and appeared to have the greatest oxida-

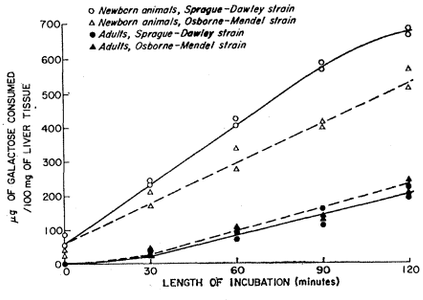

Fig. 2. The rate of galactose consumption by liver tissue from adult and neonatal rats of two different laboratory strains. The tissue was prepared as described in Fig. 1, except that the time of incubation was varied, duplicate incubations being run for "0", 30, 60, 90, and 120 minutes.

Table 1. The amount of galactose-1-C^{14} oxidized to $C^{14}O_2$ by 100 mg of rat liver tissue in 1 hour, calculated from the amount of $C^{14}O_2$ liberated. The tissue was obtained from normal rats and prepared and incubated as described in Fig. 1, except that pooled liver tissue was used, and incubation flasks were modified as previously described (*9*) to permit collection of CO_2 at the end of the incubation. Initial galactose concentration was 50 mg per 100 ml (as in Fig. 1), and the medium contained 280,000 counts per minute of galactose-1-C^{14}, at a counting efficiency of 57 percent. Evolved CO_2 was quantitatively collected and counted (*9*) and results were calculated on the basis of the assumption that each molecule of radioactive CO_2 released represented the oxidation of a molecule of galactose. Values represent averages of triplicate determinations on pooled tissue from three litters of fetuses and newborn rats and from three adult rats.

Age of animals (days)	Amount of galactose-1-C^{14} oxidized (μg)
−7	90.5
−1	21.2
+1	29.1
+20	20.2
+60	13.8

tive capacity of any of the tissues studied.

All the data are reported in terms of the wet weight of the liver tissue. The calculations could also be performed on the basis of milligrams of liver protein, but this would only accentuate the differences between newborn and adults, since the protein content per unit of wet weight is 30 percent greater in the liver of adults than in newborn liver (*4*). It should be noted that liver tissue from a full-term fetus or newborn is between 30 and 40 percent hematopoietic tissue (*5*). It is difficult to assess the bearing of this circumstance on the interpretation of the data presented here, as well as on the interpretation of the work describing Gal-transferase activities in newborn liver (*2*).

The observations reported here suggest that in normal liver the formation of UDPGal by way of the pyrophosphorylase pathway may contribute little to the capacity of the liver to metabolize galactose, especially since Isselbacher has shown that the relative activities of transferase and UDPGal-pyrophosphorylase do not vary with age, a constant ratio of 5:1 being maintained from birth (when activities are low) to adulthood (when both activities are high) (*2*).

That UDPGal-pyrophosphorylase does not play a role in galactosemic subjects can be concluded from the inability of

most postpubertal patients with this disease to metabolize galactose (*3*).

In spite of the evidence that the Gal-transferase and UDPGal-pyrophosphorylase activity of liver tissue increases with age, the data presented here demonstrate a decrease with age in the ability of liver tissue to utilize galactose. One possible explanation for the discrepancy between the enzyme levels and the activity of whole tissues, is that these particular enzymes are not rate-determining for the series of reactions converting galatose to glucose. The rate-limiting step may be the initial phosphorylation of galactose by the enzyme, galactokinase, according to the reaction,

$$\text{Galactose} + \text{adenosine-triphosphate (ATP)} \longrightarrow \text{adenosinediphosphate} + \text{Gal-1-P},$$

and, indeed, Kirkman and Kalckar (*6*) have presented some evidence that this is the case for red blood cells.

If the diminished rate of galactose utilization demonstrated here in the adult liver were, in fact, due to a decrease in the phosphorylation of galactose (whether because of lowered galactokinase activity or lowered ATP availability, or for other reasons) this would imply a decreased rate of galactose-1-phosphate production in the adult, and would be consistent with the speculation that the accumulated galactose-1-phosphate is the toxic agent.

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References and Notes

1. H. M. Kalckar, E. P. Anderson, K. J. Isselbacher, *Biochim. Biophys. Acta* **20**, 267 (1956).
2. K. J. Isselbacher, *Science* **126**, 652 (1957).
3. S. Segal, A. Blair, Y. J. Topper, *ibid.* **136**, 150 (1962). Since the appearance of this report, studies on three additional galactosemic adults have shown that these patients were virtually unable to metabolize galactose.
4. B. Combes and G. S. Stakelin, *J. Clin. Invest.* **41**, 750 (1963).
5. M. Spatz, personal communication.
6. H. N. Kirkmann, H. M. Kalckar, *Ann. N.Y. Acad. Sci.* **75**, 274 (1958).
7. All animals were obtained from the animal colony of the National Institutes of Health, Bethesda, and had been maintained on conventional rat rations, under ordinary conditions.
8. The technique for assaying galactose was devised in this laboratory, and exploits the properties of the enzyme, galactose oxidase, described by J. A. D. Cooper, W. Smith, M. Bacila, H. J. Medina, *J. Biol. Chem.* **234**, 445 (1959); and further characterized by C. Avigad, D. Amaral, C. Asensio, B. L. Horecker, *J. Biol. Chem.* **237**, 2736 (1962); and B. W. Agranoff, N. Radin, W. Suomi, *Biochim. Biophys. Acta* **57**, 194 (1962). A detailed description of the technique, with useful applications to blood, incubation media, and other fluids of biological interest, is in preparation.
9. S. Segal, A. Blair, A. N. Weinberg, *Metab. Clin. Exptl.* **9**, 1033 (1960).

26 August 1963

Blood Pressure Changes during Human Sleep

Abstract. *Systolic blood pressure measurements were made on normal human subjects throughout entire nights of natural sleep and were correlated with cyclical changes in electroencephalographic patterns. During the recurrent rapid-eye-movement phase of sleep mean blood pressure levels were found to be generally higher, and the minute-to-minute variability of level was much greater than during the remainder of sleep.*

The distinctive and regularly recurring electroencephalographic (EEG) phase of sleep [rapid-eye-movement (REM) sleep] associated with human dreaming (*1*), is accompanied by changes in many physiological systems (*2*). As part of a broader investigation of vegetative changes during human sleep (*3*) the present study tests the hypothesis that periodic changes in blood pressure are concomitant with the alternating EEG phases of sleep, though existing evidence did not predict the nature of the changes. The very similar phase of sleep in the cat is reported to be accompanied by lowered blood pressure levels (*4*), while it was much earlier suggested that marked and sudden increases of blood pressure in response to dreaming might account for cardiovascular catastrophes during sleep (*5*). Previous investigations of blood pressure during human sleep (*6*) consistently report a fall at sleep onset and a gradual rise toward morning, but do not relate the detailed time course of blood pressure to the EEG cycle. To attempt such a correlation requires frequency and reliability of measurements which are difficult to reconcile with the conditions of natural sleep.

To our knowledge there is no existing method of blood pressure measurement, including the one employed here, which is ideally suited to these requirements and conditions. However, certain modifications of the conventional indirect blood pressure measurement provided a technique which did demonstrate significant changes in relation to the EEG patterns of sleep. This technique involved the recording of pulse sounds through a microphone taped over the posterior tibial artery at the ankle, while an ordinary blood pressure cuff upon the lower leg was automatically inflated and deflated over a 1-minute cycle. As compared with measurements from the arm, this location is much less disturbing to the sleeper, and the range or frequency of positional changes with reference to heart are smaller, though still not negligible. Pressure levels within the cuff were recorded on the polygraph by means of an electrical manometer (Beckman Infratron FBR-2A) which produces a series of coded pulses as in Fig. 1. As shown in the same figure, this allows an estimate of systolic pressure as that coinciding with the first sound impulse, and of diastolic level as the point of abrupt diminution in intensity of this impulse. In practice the diastolic measure was not sufficiently reliable to be used.

Subjects were normotensive "normal control volunteers" (*7*), five male and seven female, ranging in age from 18 to 26. The first one or two nights for each subject were somewhat broken by periods of wakefulness and were not used, but after adaptation the EEG records were typical of those obtained under laboratory conditions, and the subjects generally denied awareness of the inflating cuff from sleep onset until final awakening.

For one night of uninterrupted sleep from each subject the time course of

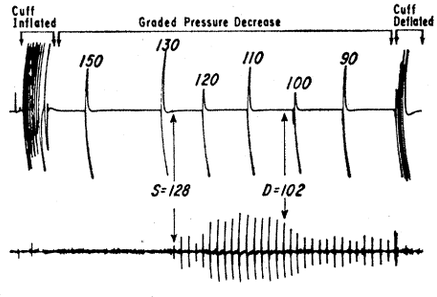

Fig. 1. Ilustration of one cycle of blood pressure recording by the method described; portion of the cycle shown was of 55 seconds duration. Upper trace: signal from manometer, showing phases of inflation cycle and graded pressure decrements. Lower trace: signal from microphone over posterior tibial artery, showing estimates of systolic (*S*) and diastolic (*D*) blood pressure.