Morse, E. M. Lederberg and J. Lederberg for the generous supply of *E. coli* mutants and for helpful discussions. I am also indebted to H. M. Kalckar for helpful suggestions made during the course of this work.

4. Abbreviations: ADP and ATP, adenosine di- and triphosphate, respectively; G-1-P, α-glucose-1-phosphate; Gal, D-galactose; Gal-1-P, α-galactose-1-phosphate; PP, inorganic pyrophosphate; UDPG, uridine diphosphoglucose; UDPGal, uridine diphosphogalactose; UTP, uridine triphosphate.
5. R. Caputto, L. F. Leloir, R. E. Trucco, *Enzymologia* 12, 350 (1948); R. Caputto *et al.*, *J. Biol. Chem.* 184, 333 (1950); H. M. Kalckar, B. Braganca, A. Munch-Petersen, *Nature*, 172, 1036 (1953).
6. K. Kurahashi and E. P. Anderson, unpublished.
7. A. Munch-Petersen, H. M. Kalckar, E. E. B. Smith, *Kgl. Danske Videnskab. Selskab Biol. Medd.* 22, No. 7, 3 (1955).
8. E. S. Maxwell, H. M. Kalckar, R. M. Burton, *Biochim. et Biophys. Acta* 18, 445 (1955).
9. J. Lederberg, *Methods in Medical Research* 3, 5 (1950).
10. P. M. Nossal, *Australian J. Exptl. Biol.* 31, 583 (1953).
11. K. Kurahashi, unpublished observation.
12. The C^{14}-labeled galactose was synthesized by H. Isbell and his coworkers at the National Bureau of Standards.
13. H. M. Kalckar, E. P. Anderson, K. J. Isselbacher, *Biochim. et Biophys. Acta* 20, 262 (1956).
14. C. Yanofsky, in *Enzymes: Units of Biological Structure and Function*, O. H. Gaebler, Ed. (Academic, New York, 1956), p. 147.
15. M. L. Morse, E. M. Lederberg, J. Lederberg, personal communication.

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Studies on Metabolism of Carbon-14-Labeled Galactose in a Galactosemic Individual

It is frequently reported (*1*) that in congenital galactosemia only about 60 to 80 percent of the galactose administered can be accounted for on the basis of urinary excretion. The fate of the remaining galactose has, so far, been virtually unknown. However, Schwartz *et al.* (*2*) have recently demonstrated that galactose administration to galactosemic children brings about a significant accumulation of a hexose phosphate identified as galactose-1-phosphate. This poses the question whether the galactose retained in the body as galactose-1-phosphate might not account for the major part, if not the total, of the fraction of the galactose that is not excreted.

We have found (*3–5*) that in hemolysates, as well as in liver homogenates (*6*) from galactosemic subjects, the enzyme that catalyzes the metabolic step immediately succeeding galactose-1-phosphate formation (galactose-1-phosphate uridyl transferase) is defective or totally absent.

We were therefore interested in studying the galactose metabolism in man with special reference to: (i) galactose-1-phosphate accumulation and (ii) residual metabolism beyond the galactose-1-phosphate stage. Concerning the latter problem, it was felt that although enzyme studies reveal a defect of major proportion, a study of galactose metabolism in the intact human organism might detect the presence of appreciable metabolism beyond simple phosphorylation. Highly sensitive methods for detecting the conversion of galactose to the glucose of glucose derivatives were based on two principles: (i) The use of C^{14}-labeled galactose and (ii) the trapping of galactose as a glucosiduronic acid. The latter principle was used for several reasons.

Studies during the last few years have shown that the irreversible conversion of glucose to glucosiduronic acids involves the very same uridine nucleotides that are operating in the conversion of galactose to glucose derivatives (*7*). Moreover, the conversion of galactose and glucose compounds to alcohol glucosiduronic acids gives rise to compounds that can readily be isolated as crystalline precipitates.

Galactose-1-C^{14} (*8*) was administered intravenously over a period of 30 minutes to a 24-year-old male with galactosemia in a dose of 5 μc. In this experiment, 1 g of nonisotopic galactose was added to the galactose-1-C^{14} being infused. Concurrent with the administration of the isotope, the subject ingested 1 g of menthol over a period of 24 hours. Urine was collected at 2-hour intervals during this time. From each collection, menthyl glucosiduronic acid was isolated as the ammonium salt (*9*) and purified (*10*).

An aliquot of the urine was taken prior to isolation, acidified, extracted with redistilled ether, and total menthyl glucosiduronic acid was determined on the ether extract by the orcinol reaction using the conditions described by Dische (*11*). The purified menthyl glucosiduronic acid was counted in solution in a Packard Tri-Carb liquid scintillation spectrometer. A sample of the substrate galactose-1-C^{14} was counted in the same way.

From the radioactivity of the pure menthyl glucosiduronic acid and the quantity of the compound excreted, total counts were calculated. From this figure and the counts administered as galactose-1-C^{14}, the percentage conversion of the substrate to glucosiduronic acid was computed. Urea was isolated for the purpose of sampling the CO_2 pool. Urinary galactose was measured by the method of Nelson (*12*) as the reducing sugar remaining after treatment of the urine with glucose oxidase (*13*). Galactose and galactose-1-phosphate in blood were detected by indirect methods. No detectable counts were found in the blood plasma. However, in the erythrocytes, appreciable amounts of counts were found (see Table 1).

The presence of galactose-1-phosphate was inferred for the following three reasons. (i) The C^{14}-labeled material was confined to the erythrocytes, with no radioactive material present in the plasma fraction. Free galactose would be distributed fairly evenly between the two fractions. (ii) The reported observation of galactose-1-phosphate accumulation in the erythrocytes from congenital galactosemia has been reported (*2, 3*). (iii) Enzymatic assay, although it was too low to be considered quantitative, revealed traces of galactose-1-phosphate (*4*).

Excreted galactose and glucosiduronic acid were measured as described. The amount of suspected galactose-1-phosphate present in the relatively small blood specimens was too minute to isolate. The radioactivity measurements were therefore performed directly on small samples of crude filtrates, and corrections for self-absorption were made. Counts for a known sample of C^{14}-labeled galactose were taken under identical conditions—that is, as an internal standard. In this way counts could be expressed as micromoles of galactose.

Filtrates from plasma were found to be nonradioactive, whereas filtrates from erythrocytes showed distinct radioactivity. The latter could not be attributed to free galactose because galactose would distribute itself freely between plasma and cells. It was therefore classified as "cellular" galactose, for any cellular incorporation of radioactivity would figure on the balance as galactose retained in the body. This is actually the essential term in the balance. From the studies by Schwartz *et al.* (*2*) as well as those by us (*5*), it seems likely that all of the cellular galactose is identical with galactose-1-phosphate.

The distribution of galactose which was found over a period of 4 hours is shown in Table 1. As can be seen, out of the 1 g of galactose administered, 75 to 80 percent was not metabolized beyond the galactose-1-phosphate stage, 3 percent was metabolized to the glucosiduronic acid stage, and 20 to 25 percent was not accounted for. The latter fraction was probably metabolized to carbon dioxide or lactic acid but diluted by carbon from the general carbohydrate pool so as to escape detection, for the urea

Table 1. Balance of galactose compounds (after the infusion of 1 g of galactose and 5 μc of galactose-1-C^{14} to a 24-year-old male with galactosemia).

Item	Amount (mg)
Galactose excreted in urine	700
Galactose metabolized to glucosiduronic acid	30
Galactose accumulating as "cellular" galactose (galactose-1-phosphate and so forth)	50 to 100*

* All tissues with the exception of muscle and bone.

contained no C^{14}. A possible loss by excretion through the gastrointestinal tract cannot be excluded.

We believe that the galactose was not only metabolized to glucosiduronic acid but that most of the remaining 200 mg of galactose was generally metabolized. This belief is partly based on an experiment in which only 1.5 mg of galactose-1-C^{14} (undiluted) was administered. In the afore-mentioned experiment, it was established that the galactosemic organism is able to metabolize 30 mg of galactose to glucosiduronic acid. One would therefore expect that if the latter pathway were the only one in operation, a minute amount of galactose, such as the 1.5 mg of C^{14}-labeled galactose that was administered in a separate experiment, would readily be completely converted to glucosiduronic acid. However, the experiment showed that only 6.5 percent of this amount appeared as glucosiduronic acid. The most reasonable assumption is that the rest went through the pathway of glucose-6-phosphate and hence escaped detection by high isotope dilution. These considerations probably also apply for the experiment in which 1 g of galactose was administered.

The special value of the isolation and analysis of the C^{14}-labeled menthyl glucosiduronic acid is manifold. (i) It demonstrates directly that galactose can definitely be metabolized in the galactosemic organism. (ii) It poses a question as to whether the block of the galactose-1-phosphate uridyl transferase is complete, for it is known from earlier studies that conjugated glucuronic acid arises from hexoses through the uridine nucleotides (*7*).

That the normal pathway, or a very closely related accessory one (compare *14*), was in operation in this case is also supported by the fact that the distribution of labeled carbon in the glucuronic acid moiety was identical with that of the galactose administered—that is, it was confined to carbon atom 1 (*15*).

A normal adult person is able to metabolize 20 to 25 g of galactose within 12 hours (*16*), whereas a galactosemic person is able to metabolize 150 to 200 mg at the most. This means that a galactosemic subject has retained only about 1 percent of the capacity of a normal person with respect to galactose metabolism. This is a somewhat lower than, but probably in the order of magnitude as, that found *in vitro* by enzymatic assays on liver tissue obtained from the same patient (*6*). It was estimated that less than 4 to 8 percent of the normal activity of the galactose-1-phosphate uridyl transferase was retained in the galactosemic liver. Alternatively, the activity could be attributed to an incorporation enzyme related to galactose-1-phosphate uridyl transferase. The red cells from the same subject showed no detectable amounts of galactose-1-phosphate uridyl transferase.

The values obtained from *in vitro* and *in vivo* studies definitely fall into the same order of magnitude. The *in vivo* method is for this particular purpose as sensitive as the *in vitro* technique. Both methods may give the approximate residual galactose-1-phosphate uridyl transferase present in the galactosemic subject. However, it is quite naturally not possible to rule out the existence of a closely related accessory exchange mechanism involving galactose-1-phosphate, which is replacing the normal pathway in congenital galactosemia.

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References and Notes

1. H. H. Mason and M. C. Turner, *Am. J. Diseases Children* 50, 359 (1935); E. H. Townsend, Jr., H. H. Mason, P. S. Strong, *Pediatrics*, 7, 760 (1951); D. J. Bell, *Biochem. J. (London)* 30, 1612 (1936); E. Bruck and S. Rapoport, *Am. J. Diseases Children* 70, 267 (1945).
2. V. Schwartz *et al.*, *Biochem. J. (London)* 62, 40 (1956).
3. H. M. Kalckar, E. P. Anderson, K. J. Isselbacher, *Proc. Natl. Acad. Sci. U.S.* 42, 49 (1956).
4. K. J. Isselbacher *et al.*, *Science* 123, 635 (1956).
5. H. M. Kalckar, E. P. Anderson, K. J. Isselbacher, *Biochim. et Biophys. Acta* 20, 262 (1956).
6. E. P. Anderson, H. M. Kalckar, K. J. Isselbacher, *Science*, this issue.
7. G. J. Dutton and I. D. E. Storey, *Biochem. J. (London)* 57, 275 (1954); J. L. Strominger *et al.*, *J. Am. Chem. Soc.* 76, 641 (1954).
8. Galactose-1-C^{14} was obtained through the courtesy of H. Isbell, National Bureau of Standards.
9. R. T. Williams, *Biochem. J. (London)* 34, 272 (1940).
10. F. Eisenberg, Jr., and S. Gurin, *J. Biol. Chem.* 195, 317 (1952).
11. Z. Dische, *ibid.* 204, 989 (1953).
12. N. Nelson, *ibid.* 153, 375 (1944).
13. E. R. Froesch and A. E. Renold, *Diabetes* 5, 1 (1956).
14. E. E. B. Smith, A. Munch-Petersen, G. Mills, *Nature* 172, 1061 (1953).
15. F. Eisenberg, Jr., and K. J. Isselbacher, unpublished.
16. H. J. Deuel, *Physiol. Revs.* 16, 173 (1936).

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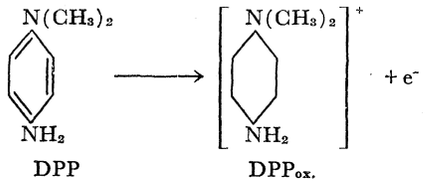
Oxidation of N,N-Dimethyl-p-phenylenediamine by Serum from Patients with Mental Disease

In investigations carried out at the Långbro Mental Hospital in 1955, it was found that fresh blood serum obtained from patients with mental disease, including schizophrenic, manic depressive, and senile psychoses (*S*-serums) had the capacity to oxidize N,N-dimethyl-*p*-phenylenediamine (DPP) more rapidly than fresh serum obtained from healthy control subjects (*N*-serums). Further investigations to determine the incidence of this reaction in nonmental diseases and to explore the possibility of using it as a diagnostic test for mental disease are in progress. This report describes the biochemical background that underlies this reaction (*1*).

Mild oxidation of DPP leads to the formation of a free radical of a semiquinone type (see for example, *2*):



DPP DPP$_{ox.}$

This substance has an absorption maximum at 552 mμ and is known as Wurster's red.

The addition of DPP (*3*) to fresh serum from healthy control subjects is followed by a lag period of approximately 5 minutes, during which there is but slight increase in the extinction at 552 mμ. A sudden acceleration in color development then ensues, with a linear increase in extinction. In contrast, the lag period in development of the red color is much shorter or absent with fresh *S*-serum (Fig. 1).

Prolonged exposure to room air, gentle aeration, or dialysis against 0.9-percent NaCl increases the capacity of *N*-serum to oxidize DPP so that it equals or approaches the rate characteristic of *S*-serum. This suggests that one or several reducing substances of low molecular weight which are present in *N*-serum are quantitatively diminished or absent from *S*-serum.

The DPP-oxidizing substance in both serums is heat labile and not dialyzable. It therefore appears to be a catalytically active protein.

The ability to oxidize DPP disappears when serum is dialyzed against a citrate-phosphate buffer of *p*H 3. Following removal of buffer ions by dialysis against 0.9-percent NaCl, this activity can be restored by addition of a low concentration of Cu^{++} ions but not by Fe^{+++} ions. This finding suggested that ceruloplasmin, a serum oxidase containing copper, which was first isolated by Holmberg and Laurell (*4*), might be involved. Ceruloplasmin constitutes the copper-binding protein of normal serum. It contains about 95 percent of the total serum copper (*5*) and is also capable of catalyzing the oxidation of *p*-phenylenediamine.

Evidence suggesting that catalytic oxidation of DPP by serum is attributable to ceruloplasmin was provided by finding that this activity is precipitated with $(NH_4)_2SO_4$ in the same fraction as is ceruloplasmin. Furthermore, DPP oxi-