similarity to the hybrid substance described by Irwin which occurs in doves (*7*), and, more particularly, to the extensive observations of Cohen on some of the red cell antigens in the rabbit in which genetic interaction has been amply demonstrated (*8*). These results may also be relevant to the problem of heterotic vigor and illustrate how this phenomenon might on occasion be due to the presence, in the heterozygote, of a substance not possessed by either homozygote (*9, 10*).

A. G. BEARN
E. C. FRANKLIN
Rockefeller Institute, New York

References and Notes

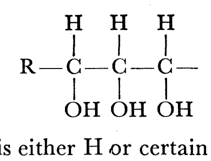
1. O. Smithies, *Biochem. J.* 61, 629 (1955).
2. G. C. Ashton, *Nature* 180, 917 (1957).
3. O. Smithies and N. F. Walker, *ibid.* 176, 1265 (1955); F. Galatius-Jensen, *Acta Genet. et Statist. Med.* 7, 549 (1957).
4. O. Smithies and N. F. Walker, *Nature* 178, 694 (1956).
5. M. F. Jayle and J. Tonnelat, *Bull. soc. chim. biol.* 38, 343 (1956); S. Guinaud, J. Tonnelat, G. Boussier, M. F. Jayle, *ibid.* 38, 329 (1956).
6. A detailed report of these findings is in preparation.
7. M. R. Irwin, *Advances in Genet.* 1, 133 (1947).
8. C. Cohen, *J. Immunol.* 80, 73 (1958).
9. J. B. S. Haldane, *Proc. Roy. Soc.* (*London*) B144, 217 (1955).
10. These studies were supported in part by a grant-in-aid (A-1542) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

31 March 1958

Isotope Effect in Oxidation of D-Mannitol-2-C[14] by Acetobacter suboxydans

Previous reports from this laboratory have described the preparation of D-mannitol-1-C[14], its oxidation by *Acetabacter suboxydans,* and the carbon-14 assay of the resulting D-fructose-1,6-C[14] (*1–3*). Because the two halves of the mannitol molecule are stereomerically identical, oxidation by *A. suboxydans* can take place at either carbon 2 or carbon 5. It was pointed out that "if oxidation took place without either an isotope effect or breakdown of the D-mannitol and resynthesis of the fragments, the derived D-fructose would be labeled equally and exclusively at carbons 1 and 6" (*3*). The early work showed that, within the error of measurement, D-fructose-1,6-C[14] was, indeed, labeled equally and exclusively at carbons 1 and 6.

Oxidation of glycitols by *A. suboxydans* is specific for the group



where R is either H or certain other substituents. Presumably, the enzyme responsible for the oxidation forms an intermediate complex that involves the three carbon atoms indicated. The presence or absence of an isotope effect in the oxidation of a labeled D-mannitol should provide information concerning the rate-determining step, and hence, the mechanism of reaction.

In a more extensive study of a possible isotope effect, both 1-C[14]- and 2-C[14]-D-mannitol have been oxidized by *A. suboxydans.* The distribution of carbon-14 in the resulting D-fructose-1,6-C[14] and D-fructose-2,5-C[14] has been determined by several chemical methods, selected to avoid isotope effects in the analysis.

The results, summarized in Table 1, confirm the earlier radioactivity analysis of D-fructose-1,6-C[14]; no isotope effect was detected. However, a small disproportionation in the distribution of carbon-14 was found for D-fructose-2,5-C[14]. This indicates that, in the oxidation of D-mannitol-2-C[14] by *A. suboxydans,* there is a small isotope effect, and oxidation is slightly less rapid at carbon 2 (C^{14}) than at carbon 5 (C^{12}).

Because of the extensive use of C[14]-labeled products in biochemical studies, the demonstration of an isotope effect in a biological oxidation is particularly significant. Although the effect is small, it shows the need for caution in the use of biological oxidations for determining the distribution of carbon-14 in carbohydrates.

All of the compounds listed in Table 1 were recrystallized to constant radioactivity, and samples were assayed in solution (*4*).

HARRIET L. FRUSH
LORNA J. TREGONING
Division of Chemistry, National Bureau of Standards, Washington, D.C.

References and Notes

1. This is part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the Division of Research of the U.S. Atomic Energy Commission.
2. H. S. Isbell and J. V. Karabinos, *J. Research Natl. Bur. Standards* 48, 438 (1952).
3. H. L. Frush and H. S. Isbell, *ibid.* 51, 167 (1953).
4. A. Schwebel, H. S. Isbell, J. D. Moyer, *ibid.* 53, 221 (1954).

7 April 1958

Table 1. Radioactivity analysis of D-fructose-1,6-C[14] and D-fructose-2,5-C[14].

Compound assayed	Carbon atoms of original D-fructose	Radioactivity after successive recrystallizations (μc/mmole)	Av. % of original radio-activity
Analysis of D-fructose-1,6-C[14]			
D-Fructose-1,6-C[14]	1, 2, 3, 4, 5, 6	2.62*	100
Potassium D-arabonate	2, 3, 4, 5, 6	1.32*	50.4
"D-Glucose" phenylosotriazole†	1, 2, 3, 4, 5, 6	4.35, 4.33, 4.34	100
4-Formyl-2-phenylosotriazole‡	1, 2, 3	2.15, 2.17, 2.16	49.8
Dimedon-formaldehyde compound§	6	2.17, 2.18	50.1
Analysis of D-fructose-2,5-C[14]			
D-Fructose 2,5-C[14]	1, 2, 3, 4, 5, 6	1.93, 1.96, 1.93	100
"D-Glucose" phenylosotriazole†	1, 2, 3, 4, 5, 6	1.94, 1.90, 1.91	
4-Formyl-2-phenylosotriazole‡	1, 2, 3	0.93, 0.94, 0.93	48.4
Erythritol	3, 4, 5, 6	0.97, 0.98, 0.99	51.4
Erythritol tetrabenzoate	3, 4, 5, 6	1.01, 1.00, 0.99	
Dimedon-formaldehyde compound§	6	none	

* Value taken from Frush and Isbell (*3*). ‡ D-*arabino-Hexose* phenylosotriazole. ‡ Phenyl-2*H*-1,2,3-triazole-4-carboxaldehyde. § 2,2′-Methylenebis(5,5-dimethyl-1,3-cyclohexanedione).

Iodine-131 Fallout in Bovine Fetus

Iodine-131 from radioactive fallout is concentrated in thyroid glands of grazing animals (*1, 2*) and the distribution suggests that I^{131} spreads over the hemisphere in which it is released (*2*). A study of the fallout of I^{131} in the thyroids of fetal animals will serve two purposes: (i) It will be helpful in evaluating the maximum biological accumulation of radioactive fallout; and (ii) it might give useful information regarding iodine distribution during pregnancy.

Gorbman *et al.* (*3*) injected I^{131} into two cows during the ninth month of pregnancy, sacrificed them 24 hours later, and studied the I^{131} distribution. They found that the I^{131} concentrations in the thyroid of the fetuses were 6 to 7 times those of the dams. Autoradiographs showed uniform distribution of radioactivity in fetal glands but nonuniform concentrations in the adult thyroids. Wolff *et al.* (*4*) showed that bovine fetuses began to concentrate iodine at 53 days of gestation and that the amount of iodine increased rapidly after the fifth month.

Barnes *et al.* (*5*) have studied I^{131} distribution in pregnant sheep which were chronically fed the radioisotope. They showed that the near-term fetal thyroid I^{131} concentration was 1 to 2 times that of the dam.

Part of one lobe of the thyroid gland from each of 40 pregnant cows was removed along with the laryngothyroid

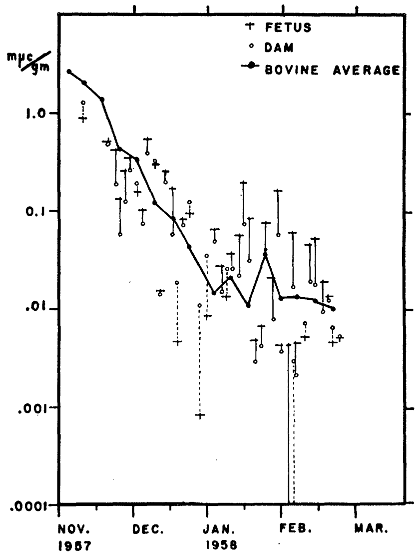


Fig. 1. Semilogarithmic average course of I^{131} fallout concentrations in thyroids of 400 arbitrarily selected cattle (solid circles). The open circles and crosses represent thyroid I^{131} concentrations of pregnant cows and of their fetuses. The solid vertical lines connecting circles and crosses represent cases in which I^{131} of the fetus exceeded that of the dam. A broken line is used when I^{131} of the dam exceeded that of the fetus.

areas of their fetuses. Whole thyroid weights were used as the best index of fetal age because this was the only accurate index of fetal size that could be made in our laboratory. Accurate histories of the animals were not available, but it is thought that most of the adult animals had grazed within 200 miles of Memphis, Tenn., and that they were kept in feed lots less than 1 week.

Iodine-131 was determined as described previously (*2*). Two fetal-dam combinations are not included in the graphs because the amounts of I^{131} were too small to measure accurately.

Figure 1 compares the I^{131} in thyroids of bovine fetuses and their dams with the curve showing concentrations in thyroids of other cattle from the same general area. The curve of average I^{131} content was determined from 400 thyroids by measuring the concentration of thyroid I^{131} in 7 to 10 unselected cattle slaughtered every Monday, Wednesday, and Friday. The average values for each week were plotted. The individual maxima and minima for each week differed by one logarithm scale during the period November to 15 December, after which the range increased to 1.4 log scales. The increased range was primarily due to relatively higher maxima after 1 January. The slope of the average curve from November to January suggests an average effective half-life of approximately 8 days for the two months following the nuclear tests conducted in the fall of 1957. After 1 January the change in slope of the average curve was probably due to new fallout. The fetal thyroid I^{131} concentrations all lie within the maximum range of normal adult animals.

Figure 2 shows the F/D ratio—that is, (fetal I^{131}/g of thyroid)/(dam I^{131}/g of thyroid)—versus the fetal thyroid weight [intra-uterine ages were determined according to the method of Nichols *et al.* (*6*)]. Eighty-five percent of the 7- to 9-month fetuses had greater I^{131} concentration in their thyroids than did their dams. Only 68 percent of the 6- to 7-month fetuses had more I^{131} in their thyroids than did their dams. These data suggest a progressive increase in F/D ratio as the fetuses matured; however, the points are probably not a two-variable normal distribution. The maximum F/D ratio found in these animals is not as great as that found 24 hours after I^{131} injection into pregnant cattle (*3*). This difference in F/D ratio may be related to differences in I^{131} exposure or to metabolic differences between the cattle used in the injection experiments and the animals reported here.

If any relation exists between the thyroid concentration of I^{131} and intra-uterine age as a function of the age of the fallout, it is not revealed by these data.

Chemical iodine has not been determined in the glands reported here, but the data of Wolff *et al.* (*4*) may be comparable. From their data it can be calculated that between 6 and 7 months of gestation, the bovine fetal thyroid had approximately one-half the concentration of I^{127} (0.55 to 0.7 mg/g of gland) as the normal cow (1.5 mg/g). Figure 2 suggests that at this same intra-uterine age (6 to 7 months) the average fetal thyroid had a slightly greater I^{131} concentration than the gland of the fetus' dam. The values for I^{127} concentration in newborn calves overlapped with the range for normal cows (*4*), but near term (7 to 9 months) I^{131} concentration in the fetal thyroid approached twice that in the dam's thyroid.

One interpretation of such results is that approximately one-half of the thyroid iodine in the dams had turned over in 6 weeks, but it may have turned over more than once. This follows if we consider the near-term fetal thyroid specific activity to approach a maximal value and if the specific activity of the adult glands remained approximately one-half that of the fetuses while there was no new I^{131} increment. This would suggest that some of the thyroid iodine pools may turn over rapidly and other pools may turn over much more slowly. This might mean that a large fraction of the thyroid iodine in the dam may be almost static. Similar differences in thyroid iodine pools were suggested by Stanbury *et al.* (*7*) and by autoradiographic studies by Gorbman *et al.* (*3*). Those cases near term in which the F/D ratio is less than 1 may illustrate dams removed from fallout, and in some cases the fractional rate of turnover of large slow pools may more nearly approximate that of the fast pools (*8*).

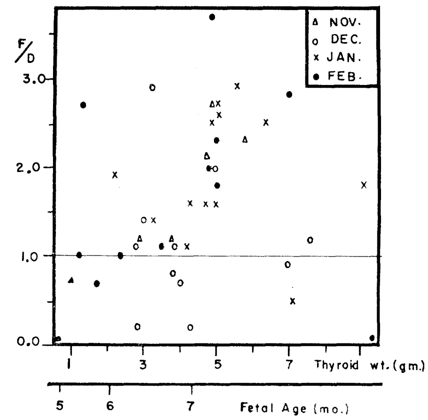


Fig. 2. (Thyroid I^{131} concentration of fetus)/(thyroid I^{131} concentration of dam) versus weight of fetal thyroid. These data represent the same animals illustrated individually in Fig. 1. The four types of points (triangles, open circles, crosses, and solid circles) show the month in which the animals were slaughtered, and this helps to locate the pairs on Fig. 1. The approximate fetal ages were derived from the thyroid weights (*3*). Thyroids of newborn calves were reported to average 5 g (*4*).

Note added in proof. Forty-two additional fetal-dam pairs were studied over the succeeding 4 months. Fourteen of these were in the fourth or fifth month of gestation with F/D ratios less than 1 in 12 of the fourteen pairs; 20 specimens were approximately at the 6-month stage, with an average ratio of 1.7 (± 1.0). Eight were in the last 3 months of gestation, with F/D ratios averaging 2.7 (± 2.2); one of these had a ratio less than 1, and one a ratio of 7.

L. Van Middlesworth

Department of Physiology, University of Tennessee, Memphis

References and Notes

1. L. Van Middlesworth, *Nucleonics* 12, No. 9, 56 (1954); C. L. Comar, B. F. Trum, U. S. G. Kuhn, III, R. H. Wasserman, M. M. Nold, J. C. Schooley, *Science* 126, 16 (1957); A. H. Wolff, *Public Health Repts.* 72, 1121 (1957).
2. L. Van Middlesworth, *Science* 123, 982, (1956).
3. A. Gorbman, S. Lissitzky, O. Michel, R. Michel, J. Roche, *Endocrinology* 51, 546 (1952).
4. J. I. Wolff, L. Chaikoff, C. W. Nichols, Jr., *ibid.* 44, 510 (1949).
5. C. M. Barnes, L. A. George, Jr., L. K. Bustad, *ibid.* 62, 684 (1958).
6. C. W. Nichols, Jr., I. L. Chaikoff, J. Wolff, *ibid.* 44, 502 (1949).
7. J. B. Stanbury, G. L. Brownell, D. S. Riggs, H. Perinetti, J. Itoiz, E. B. Del Castillo, *Endemic Goiter* (Harvard Univ. Press, Cambridge, Mass., 1954), p. 141.

8. Thyroid glands were obtained three times per week from Memphis through the cooperation of John R. Anthony. This study was supported by grants from the U.S. Atomic Energy Commission.

24 March 1958