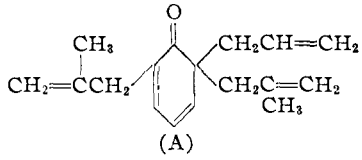
and 1-methallyloxy-2-propyl-6-isobutylbenzene (b. p. 67–68° at 0.05 mm., $n^{20}D$ 1.4915).

The results are consistent with the mechanism proposed by Hurd and Pollack[1] in which (I), for example, rearranges to the intermediate (A) which,



(A)

in turn, undergoes similar internal rearrangement to give the phenols (III) and (IV).

The possibility remains that a species such as (A) is not in the direct path between the ether (I) and the *para*-rearrangement product (III) but that (A) rearranges to (III) *via* (I). The modified Dewar mechanism for the *para*-rearrangement is, therefore, not excluded.

To settle this point, recovered starting material and products were analyzed after incomplete rearrangement of (I). Indeed, after 70% completion, infrared analysis of 10% solutions of the ethers in carbon tetrachloride using the maxima at 1050 and 925 cm.$^{-1}$ showed the recovered ether to contain only 85% of (I) and 15 ± 2% of the *rearranged ether* (II) and when (I) was heated without solvent, analysis of the recovered ether after 50% reaction showed that it had rearranged to (II) to the extent of 48%. It is likely that the (I) rearranges to (A) which can undergo reversion to (I), or rearrangement to (II). When the ether fraction recovered after 30% rearrangement of (I) was examined, however, it contained not more than 5% of the isomeric ether (II) and the composition of the phenolic products was the same as that after complete reaction.

It seems clear that the interconversion of the ethers (I) and (II) cannot provide the explanation of the formation of the large amount of the *p*-methallylphenol (IV) found in the rearrangement of the allyl ether (I). The present results strongly support, therefore, the Hurd and Pollack mechanism for the *para*-Claisen rearrangement and appear to exclude the possibility that the Dewar mechanism plays an important role in this reaction.

The rearrangement of dienone (A) to the methallyl ether (I) which is suggested by the work above, seems to be an example of a rearrangement of a new type and we hope to investigate it further.

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RECEIVED JANUARY 22, 1954

IMPORTANCE OF GLYCOLYTIC AND "OXIDATIVE" PATHWAYS IN GLUCOSE UTILIZATION BY LIVER[1]

Sir:

Bloom and Stetten[2,3] studied the formation of $C^{14}O_2$ from C^{14}-labeled glucose and lactate in tissue slices, and observed that in rat liver the yield of $C^{14}O_2$ from 1-C^{14}-glucose exceeded that from evenly labeled and from 6-C^{14}-glucose. They derived equations relating the $C^{14}O_2$ formed from the evenly and the 1-labeled glucose to that from 1-, 2- and 3-C^{14}-lactates, and on the basis of these equations concluded that glucose catabolism in rat liver does not proceed mainly *via* the Embden–Meyerhof scheme but involves the direct oxidation and decarboxylation of carbon 1 of glucose (hexose monophosphate shunt). They stated that not more than 20–25% (and possibly none) of the CO_2 arising from glucose was formed glycolytically. We have confirmed the observations of Bloom, *et al.*[2] It is shown here, however, that the derivation of their equations is questionable. When the experimental data of Bloom, *et al.*,[2] are applied to revised equations presented here, we find that at least 80% of the CO_2 derived from glucose in liver could have arisen *via* glycolysis.

Let us designate, as in ref. 2, the $C^{14}O_2$ yields from evenly labeled and 1-C^{14}-glucose as a and b, respectively, and the ratio a/b as U. It is assumed, as in ref. 2, that glucose is oxidized to CO_2 by two pathways, namely, glycolysis and the direct oxidative shunt, and that E represents the fraction of the CO_2 formed glycolytically.

If m moles of CO_2 are formed from glucose, mE moles will be formed *via* glycolysis, and $m(1 - E)$ *via* the shunt. The radioactive yield of each fraction can be represented by mE and $m(1 - E)$ multiplied by their respective molar specific activities.

If the molar specific activity of glucose is taken as 1, the molar specific activity of the $C^{14}O_2$ derived from evenly labeled glucose will be 1/6, irrespective of its mode of formation. The molar specific activity of the $C^{14}O_2$ formed from glucose-1-C^{14} by the direct oxidative path will be 1, and that formed glycolytically will be $e/2(c + d + e)$, where c, d and e represent the extent of conversion to $C^{14}O_2$ of the carboxyl, alpha and beta carbons of pyruvate (or lactate) formed from glucose. Let $R = (c + d + e)/3e$ (R is equivalent to the expression $R = (N + T + 1)/3$ in ref. 2), then the molar specific activity of the $C^{14}O_2$ formed glycolytically from glucose-1-C^{14} will be $1/6R$. Thus, the ratio, a/b, *i.e.*

$$\frac{C^{14}O_2 \text{ from evenly labeled glucose}}{C^{14}O_2 \text{ from 1-}C^{14} \text{ labeled glucose}} = \frac{\frac{mE}{6} + \frac{(1 - E)m}{6}}{\frac{mE}{6R} + (1 - E)m} = U$$

$$\text{whence } E = \frac{R(6U - 1)}{U(6R - 1)} \qquad (1)[4]$$

By using the experimental values of Bloom, *et al.*,[2] for R and U, namely, 2 and 0.6, respectively, in our equation (1), we find that $E = 0.79$, or that 79% of the CO_2 derived from glucose was formed *via* the classical glycolytic scheme.

(1) Supported by contract with the U. S. Atomic Energy Commission.

(2) B. Bloom, M. R. Stetten and D. Stetten, Jr., *J. Biol. Chem.*, **204**, 681 (1953).

(3) B. Bloom and D. Stetten, Jr., THIS JOURNAL, **75**, 5446 (1953).

(4) Compare with equation (4) of ref. (2), $U = [6RE + (1 - E)]/[6E + 6(1 - E)]$, whence $E = (6U - 1)/(6R - 1)$. E in this equation actually represents the fraction of the CO_2 formed from the *first* carbon of glucose *via* glycolysis, and does not have the meaning designated by Bloom, *et al.*[2] It should be pointed out that, while referring to the same numerical values in a subsequent paper,[3] these authors have defined E as the fraction contributed by glycolysis "to the over-all conversion of glucose to carbon dioxide," which has a different meaning and different numerical value from the E used in ref. 2 and here.

In a similar manner we have derived the following equation for the ratio ($C^{14}O_2$ from 6-C^{14}-glucose/$C^{14}O_2$ from 1-C^{14}-glucose), which we shall designate W

$$W = \frac{(E/6R)}{(E/6R) + (1 - E)}, \text{ whence}$$

$$E = \frac{6RW}{1 + W(6R - 1)} \qquad (2)$$

Again, using the experimental values of Bloom, *et al.*,[2,3] for R and W, namely, 2 and 0.3, respectively, we calculate that 84% of the CO_2 derived from glucose was formed glycolytically, a value in good agreement with that obtained from our equation (1).

In our experiments with rat liver slices, the observed ratio $U = a/b$ was higher than that reported by Bloom, *et al.*,[2] and varied between 1 and 1.8. However, these workers added lactate, acetate and gluconate, in high concentrations, to their media. Under such conditions, as shown in Table I, $C^{14}O_2$ formation from glucose is depressed, and the relative importance of the hexose monophosphate shunt is increased.

TABLE I

250-mg. rat liver slices incubated with 2.5 ml. of Krebs–Ringer bicarbonate buffer containing 55 μmoles of labeled glucose. The addition was 50 mm. each of lactate, acetate and gluconate. Gas phase 95% O_2 + 5% CO_2; incubated for 3 hours at 37°.

Label in glucose	Addition		% of C^{14} in $C^{14}O_2$	$U = a/b$	$E^a \times 100$%
even-C^{14}	None	a	2.8		
				1.2	94
1-C^{14}	None	b	2.3		
even-C^{14}	+	a	0.8		
				0.6	79
1-C^{14}	+	b	1.4		

[a] These values of E are calculated from our equation (1) using the value of $R = 2$ determined experimentally by Bloom, *et al.*[2]

Thus, in rat liver slices, over 90% of the CO_2 is derived from glucose *via* glycolysis, and even under the special conditions of Bloom, *et al.*,[2,3] about 80% of the CO_2 is formed glycolytically.

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RECEIVED JANUARY 25, 1954

IDENTIFICATION OF A FOURTH ABNORMAL HUMAN HEMOGLOBIN

Sir:

In addition to normal adult (A) and fetal (F) hemoglobins three abnormal forms (S, C and D) of human hemoglobin have been described.[1,2] We wish to report the identification of a fourth abnormal form in the erythrocytes of a child (M. M.) with an atypical anemia. Filter paper electrophoresis of hemoglobin from this individual in 0.01 M sodium barbital, pH 9.2, at room temperature revealed two components, one with the mobility of hemoglobin F and the other with a mobility very nearly that of hemoglobin C. The same result was obtained by moving boundary electrophoresis in 0.01 M Na_2HPO_4, pH 8.8, at 1.4°. Moving boundary electrophoresis in cacodylate buffer of ionic strength 0.1 and pH 6.5 at 1.4° showed a component with the mobility of hemoglobin F and a component with a mobility greater than that of hemoglobin A but slightly less than that of sickle cell (S) hemoglobin. The mobilities of hemoglobins A, S and C in this buffer are, respectively, 2.4, 2.9 and 3.2 × 10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}.[1] The component having the mobility of hemoglobin F comprised 41% of the total by electrophoretic analysis. This component was isolated from the ascending limb of a moving boundary experiment in 0.01 M Na_2HPO_4; its ultraviolet absorption spectrum was found to be that of hemoglobin F. The ultraviolet spectrum of the original specimen did not differ significantly from that of a comparable mixture of hemoglobins A and F.[3] When the original specimen was partially denatured with dilute sodium hydroxide, the amount of alkali-resistant hemoglobin recovered corresponded to the amount of the fetal electrophoretic component in the specimen. The alkali-resistant component was found to have the ultraviolet spectrum of hemoglobin F. The non-fetal component, isolated from the ascending limb of the pH 6.5 moving boundary experiment, had the color and visible spectrum of hemoglobin. The solubility of the original specimen as amorphous ferrohemoglobin in 2.58 M phosphate buffer of pH 6.8 at 25° was 1.94 g. per liter, a value similar to those of mixtures of hemoglobins A and F under the same conditions.[4] We conclude from these results that the hemoglobin specimen examined consisted of a mixture of hemoglobin F and a hitherto undescribed abnormal human hemoglobin, which we shall call hemoglobin E. It differs from all of the previously described forms in its electrophoretic behavior. Its absorption spectrum, solubility and lability to alkali denaturation are similar to those of normal adult hemoglobin. A detailed account of this work will be published later.

(1) H. A. Itano, *Science*, **117**, 89 (1953).
(2) J. V. Neel, *et al.*, *ibid.*, **118**, 116 (1953).
(3) G. H. Beaven, H. Hoch and E. R. Holiday, *Biochem. J.*, **49**, 374 (1951).
(4) H. A. Itano, *Arch. Biochem. Biophys.*, **47**, 148 (1953).

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RECEIVED MARCH 22, 1954

REACTION OF BIS-(CYCLOPENTADIENYL)-TITANIUM DICHLORIDE WITH ARYLLITHIUM COMPOUNDS[1]

Sir:

Bis-(cyclopentadienyl)-iron was first described[2] in 1951. Since then, analogous complexes of a number of other transition elements have been prepared.[3] The structure of the iron complex has

(1) This work was carried out under Contract Nonr-582(00) with the Office of Naval Research.
(2) T. J. Kealy and P. L. Pauson, *Nature*, **168**, 1039 (1951).
(3) (a) G. Wilkinson, THIS JOURNAL, **74**, 6146, 6148 (1952); **76**, 209 (1954); (b) G. Wilkinson, P. L. Pauson, J. M. Birmingham and F. A. Cotton, *ibid.*, **75**, 1011 (1953).