reaction mixture on a Dowex 1-Cl column with a 0.015 *M* HCl–0.2 *M* KCl mixture revealed several radioactive peaks. The major peak, which contained no inorganic phosphorus or adenine derivatives, gave positive reactions for keto-pentose and organic phosphorus. These fractions were treated with barium and ethanol and the precipitated barium salt washed with ethanol and dried. The pentose was characterized as ribulose by color tests and paper chromatography. After dephosphorylation with a potato phosphatase the product gave an absorption spectrum in the orcinol test identical to that given by ribulose, with peaks at 540 and 670 mμ.[2] In the cysteine–carbazole[3] test the maximum color intensity developed in 15–20 minutes with a peak at 540 mμ. This behavior is characteristic of ribulose.[4] Paper chromatography of the dephosphorylated sugar revealed only a single component. This had the same R_f as ribulose and gave the characteristic ribulose color when sprayed with an orcinol reagent[5] or the aniline phthalate reagent[6] (Table I).

TABLE I

CHROMATOGRAPHY OF THE DEPHOSPHORYLATED REACTION PRODUCT

	R_f		Color of Spot	
	Acetone–H_2O[a]	Phenol[b]	Orcinol	Aniline-phthalate
Ribose	0.60	0.56	None	Pink
Sedoheptulose	.52	...	Blue	...
Xylulose	.67	.56	Purple	...
Ribulose	.63	.63	Brown	Brown
Reaction product	.63	.62	Brown	Brown

[a] 10 parts acetone, 3 parts water. [b] Water-saturated phenol.

The composition of the reaction product isolated from the ion-exchange chromatogram is shown in Table II. It contained two equivalents of organic phosphate per mole of ribulose. For the quantitative ribulose assays the dephosphorylated sugar was used, since the behavior of the phosphate ester in these reactions has not been determined.

TABLE II

ANALYSIS OF RIBULOSE DIPHOSPHATE[a]

	μM/ml.
Total P	10.5
Inorganic P[b]	9.7
Reducing sugar[b]	5.1
Ribulose (orcinol)[b]	5.5
Ribulose (cysteine carbazole)[b]	4.7

[a] To 25 mg. of the dried barium salt in 1.8 ml. H_2O were added 0.2 ml. of 0.21 *N* H_2SO_4 and 0.04 ml. of 0.57 *M* K_2SO_4. After removal of $BaSO_4$, the solution (*p*H 5) was incubated for 60 minutes at 34° with 0.03 ml. of potato phosphatase solution. [b] After phosphatase treatment.

The rate of hydrolysis of RuDP in normal acid at 100° was found to follow first order kinetics, indicating that both acid groups were nearly equally acid labile. The half time of hydrolysis was about thirty minutes, which is similar to that of ribulose 5-phosphate. The enzyme which catalyzes the esterification reaction appears to be a phosphopentokinase; it remains to be determined whether isomerization of ribose 5-phosphate to ribulose 5-phosphate preceeds the reaction with ATP.

Ribulose diphosphate was first described by Benson as one of the early products of photosynthesis.[7] Quayle, *et al.*, have reported the formation of phosphoglycerate from ribulose diphosphate with algal extracts.[8] The results reported here are consistent with the view that RuDP is an intermediate in the carbon dioxide fixation system in which ribose 5-phosphate is the substrate.[1] The availability of substrate amounts of RuDP will facilitate further studies on the carboxylation reaction.

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THE SITE OF ATTACK BY THROMBIN ON FIBRINOGEN[1]

Sir:

Although two principal fragments are released from fibrinogen when it is activated by thrombin,[2] it has been deduced from the kinetics of clotting that only one collision is involved,[3] and it may be inferred that the fragments come from a relatively small area on the fibrinogen surface. The resultant change in electrostatic charge configuration probably sets the pattern for the subsequent polymerization. Hypothetical illustrations have been given[4] with the interaction site at one end of the rod-shaped fibrinogen molecule or on one side midway between the ends; either arrangement could explain the subsequent lateral dimerization with partial overlapping which has been postulated as the primary polymerization process.[5] We now present evidence that the site is actually midway between the ends.

Electrical birefringence measurements have been made on bovine fibrinogen in 3 *M* urea in 64% aqueous glycerol, before and after activation by throm-

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bin. The solutions of activated fibrinogen were prepared from fibrin clots dissolved in urea. The Kerr coefficients were obtained, and development and decay curves resulting from square pulses were analyzed following Benoit.[6] The decay curves provided rotary diffusion constants in agreement with those previously obtained from flow birefringence[7] and corresponding to rotation of the long axis[8] of about 600 Å. The development curves showed that at *p*H 6 the orientation was entirely attributable to induced polarization. However, at higher *p*H (7 to 10) there were contributions from permanent dipole moment. In this range, the calculated dipole moment[9] of activated fibrinogen was found to exceed that of the unactivated molecule by not more than 110 *D*.

Regardless of the value of net charge, the change in dipole moment $\Delta\mu$ accompanying an alteration of charge by Δz units at a distance x from the center of symmetry of the molecule[11] is $x\epsilon\Delta z$, where ϵ is the electronic charge. Since the loss of peptides upon activation involves[2,12-14] a Δz of 10 to 14, it follows that x is not greater than 1 to 2 Å. This represents a distance measured along the long axis, of course; components of dipole moment parallel to the short axis would not be detected in these experiments. We conclude that the site of attack by thrombin is on one side, equidistant from the ends of the fibrinogen molecule.[15]

Further details will be reported subsequently.

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(16) Proctor and Gamble Fellow in Chemistry, 1953–54.

BIOSYNTHESIS OF LEUCINE IN BAKERS' YEAST[1]

Sir:

Recent isotopic studies of the biosynthesis of leucine in micro-organisms have indicated that in yeast,[2] $CH_3C^{14}OOH$ gave rise to leucine with approximately half of its activity residing in the carboxyl carbon. In similar experiments in *Escherichia coli*[3] and *Rhodospirillum rubrum*[4], over 70% of the activity in leucine was located in the carboxyl carbon. $C^{14}O_2$ was found to be incorporated to only a limited extent into leucine in *Rhodospirillum*.[4]

In this laboratory the test organism, *Saccharomyces cerevisiae*, was obtained by isolation from a cake of Fleischmann's bakers' yeast, stock cultures being carried on malt–agar slants. To these samples, previously grown on glucose, was administered 20 mM. each of $CH_3C^{14}OCOOH$ or $CH_3C^{14}OOH$ with a specific activity of 18.5×10^5 c.p.m. per mM., as the sole carbon source in a salts–$(NH_4)_2SO_4$ medium. In the pyruvate experiment all the labeled substrate was utilized aerobically in four hours. With acetate as substrate, 39% was utilized under similar conditions. Details of these fermentations have been given elsewhere.[5]

Purity of the leucine samples, isolated from the hydrolysates of the bakers' yeast by means of Dowex-50 column chromatography,[6] was established by paper chromatography, using *sec*-butanol–NH_3 as the solvent system.[7] Various concentrations of the leucine samples were employed, and in no case were other amino acids observed. Degradation studies of this amino acid were carried out in the following manner: (1) combustion for the whole molecule; (2) ninhydrin decarboxylation for

TABLE I

RADIOACTIVITY IN THE CARBON SKELETONS OF GLUTAMIC ACID AND LEUCINE FROM YEAST GROWN ON $CH_3C^{14}OOH$ AND $CH_3C^{14}OCOOH$

Glutamic Acid		Leucine			
Radioactivity					
Carbon atom	Found,[12] %	Carbon atom	Calcd. %	Found %	Found c.p.m.[b] × 10^{-5}/mM
Acetate substrate					
1 COOH[a]	34	5′CH_3	0	0	
		5 CH_3	0	0	
2 $CHNH_2$	0	4 CH	0	0	
3 CH_2	0	3 CH_2	0	0	
4 CH_2	0	2 $CHNH_2$	0	0	
5 COOH	64	1 COOH	100	100	0.50
Total					0.50
Pyruvate substrate					
1 COOH[a]	26	5′CH_3	0	1	0.04
		5 CH_3	0	1	0.04
2 $CHNH_2$	17	4 CH	23	25	0.72
3 CH_2	19	3 CH_2	26	25	0.71
4 CH_2	0	2 $CHNH_2$	0	0	0.01
5 COOH	39	1 COOH	51	47	1.37
Total					2.89

[a] Carbon 1 of glutamic acid is lost in the proposed scheme of leucine biosynthesis. [b] Specific activity (total) is expressed as counts per minute per millimole of amino acid; the activities of individual carbon atoms are counts per minute per millimole of carbon.

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