We also distributed the hypertensin obtained with horse hypertensinogen and purified hog renin, both previously dialyzed until the reaction for chloride ion was negative and the resulting diagram was similar although not identical to *b*.

The theoretical curves placed under each distribution curve point out the complexity of of the active material being analyzed. The actual number of different components can be much greater.

SKEGGS *et al.*[12] have found that the hypertensin obtained from horse hypertensinogen and hog renin, in a chloride-free medium (hypertensin I), can be converted to a different equally pressor compound (hypertensin II), apparently through the action of an enzyme in the plasma which requires halide or nitrate for activation.

PEART[9] has found two peaks of pressor activity in his preparations using partition chromatography. One of them had 5 % of the total activity.

It is difficult to explain at present the origin and physiological importance of the hypertensins reported here but it seems fairly well established that the preparations currently employed as starting materials for the purification may consist of a complex mixture of substances with similar biological activities. This fact may explain some of the discrepancies now existing in the field.

The origin of the different pressor activities, their pharmacology and connected problems are being investigated in this laboratory.

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Phosphoglycerate formation from pentose phosphate by extracts of *Thiobacillus denitrificans*

An enzyme system has been demonstrated in *Chlorella*[1] and spinach leaves[2] which carboxylates one molecule of pentose phosphate with the formation of two molecules of phosphoglycerate (PGA). This reaction is now considered to be the primary CO_2 fixing mechanism in photosynthesis[3,4]. When pentose monophosphate is the substrate, adenosinetriphosphate (ATP) is required for the formation of ribulose diphosphate[5], the actual CO_2 acceptor in the reaction[1,6,7].

Crude extracts of the chemosynthetic autotroph, *Thiobacillus denitrificans*, have been prepared which fix $^{14}CO_2$. Fixation was increased by the addition of ATP to the system and further increased by the addition of ribose-5-phosphate (R-5-P). The addition of R-5-P in the absence of ATP only slightly increased the amounts of $^{14}CO_2$ fixed. Table I shows the effect of adding R-5-P to the extract plus ATP. The mixture was deproteinized with trichloroacetic acid and a "phosphate ester" fraction prepared by treatment of the supernatant with barium acetate and ethanol at pH 8. Barium was removed from the precipitate with Dowex-50 (H form) and the solution so obtained freeze-dried. The product was chromatographed on Whatman No. 1

paper using ethylacetate-acetic acid-water (3:3:1)[8] and radioautographs of the chromatograms made. A spot corresponding to PGA was found. It will be seen from Table I that, after correcting for the fixation in the absence of R-5-P, about 47% of the activity of the "phosphate ester" fraction was due to PGA.

TABLE I

FIXATION OF $^{14}CO_2$ BY AN EXTRACT OF *Thiobacillus denitrificans*

The system contained in a volume of 5 ml 8.6 mg enzyme N, 10 μmoles ATP; 10 μmoles R-5-P, 4.8 μmoles $NaH^{14}CO_3$ (1,500,00 c.p.m.), 30 μmoles $MgCl_2$ and 0.1 *M* tris-hydroxymethylaminomethane pH 7.1. Incubation 3 h at 30° C under oxygen-free N_2.

	c.p.m. ^{14}C fixed/ml incubation mixture		
	No R-5-P (a)	*R-5-P (b)*	*(b) − (a)*
Total	20,400	46,900	26,500
"Phosphate ester" fraction	9,500	24,700	15,200
Phosphoglyceric acid	980	8,170	7,190

The evidence that the material was PGA is as follows.

(a) It could not be separated chromatographically from authentic PGA using the solvents, *iso*propyl ether-90% formic acid (9:6)[9], methanol-ammonia (S.G. 0.88)-water (6:1:3)[10], and *tert.* amyl alcohol, *p*-toluenesulphonic acid-water (60 ml: 2 g: 30 ml, upper phase[7]).

(b) Hydrolysis with potato phosphatase[11] gave a compound which could not be separated chromatographically from authentic glyceric acid using the solvents amyl alcohol-5 *M* formic acid (1:1 upper phase)[12] and butanol-propionic acid-water, prepared according to BENSON *et al.*[13].

Glyceric acid is oxidised by periodate to CO_2, formic acid and formaldehyde, the CO_2 arising exclusively from the carboxyl group[14,15]. Periodate treatment of the glyceric acid, obtained as described above, showed that all the radioactivity was located in the carboxyl group (Table II).

TABLE II

POSITION OF ^{14}C IN GLYCERIC ACID

	c.p.m.
Total ^{14}C *	4080
Carboxyl ^{14}C **	4150

* Wet combustion[16].
** Periodate oxidation.

Recently the crude extract has been fractionated with ammonium sulphate and a fraction obtained which fixed $^{14}CO_2$ only in the presence of ATP and R-5-P; 93% of the fixed ^{14}C was recovered in PGA. In contrast to the crude enzyme the amount of $^{14}CO_2$ fixed in the presence of ATP alone was insignificant. This experiment indicated that PGA is the first stable compound formed during the carboxylation of pentose phosphate.

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