dissolved in water and tested directly; 1 ml. of this preparation had an equivalence of 0·65 μgm. barium-phosphopyridoxal. Other samples which had been boiled at *p*H 4 or 8 showed further deterioration.

Green, Leloir and Nocito[12] prepared two transaminating enzymes from pig heart, one carrying out a transamination from glutamic acid to α-ketoglutaric and the other from glutamic acid to pyruvic acid. This second enzyme is presumably the same as glutamic aminopherase of Braunstein. Green *et al.* found that the pure enzyme contained codecarboxylase as a constituent of the preparation, and it can be calculated from the figures in their paper that 1 mgm. of the purest enzyme preparation contained 0·269 γ gm. phosphopyridoxal when assayed against 'dopa' decarboxylase. Prof. Braunstein stated in a letter to us that the transamination *Q* of his preparation was approximately 18,000 compared with an activity of 29,000 for the highest attainable purity according to Lènard and Straub. Consequently the assay value of 0·18 μgm. phosphopyridoxal per mgm. enzyme (*Ea* Table 3) compares very well with that obtained by Green *et al.*

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Reversible Splitting of Glutamic Aminopherase

Two enzyme systems catalysing the transamination of amino-acids have been prepared from muscle and heart tissue and described by one of us (M.K.)[1,2]. These enzymes were termed glutamic aminopherase and aspartic aminopherase, according to their specific primary substrates[3]. The latter is easily inactivated in the initial stages of purification (dialysis, etc.), and can be reactivated by a thermostable coenzyme present in boiled tissue extracts[4]; while the former can be prepared from muscle tissue without loss of activity.

In a preliminary note[5] it was suggested that glutamic aminopherase might contain a prosthetic group less readily dissociable than that of aspartic aminopherase. Recently we obtained experimental data indicating that glutamic aminopherase prepared by the method of Lènard and Straub[6] (stage *B*) can be inactivated reversibly by acidification to about *p*H 2·8 or alkalization (in a broad range near *p*H 10–11), followed by dialysis. Part of the enzyme is inactivated irreversibly, depending upon the degree of acidity or alkalinity. The inactivated enzyme (= apo-enzyme) can be reactivated to some extent by the addition of boiled muscle (or liver) extract. The degree of reactivation ranges from 20 to 70 per cent, as shown in the accompanying table, averaging 37 per cent, and 30 per cent upon acid- and alkaline-splitting respectively.

DECREASE OR FORMATION OF PYRUVATE[6] IN μMOL. PER ML. ENZYME (ABOUT 100 LENARD–STRAUB UNITS).
COMPOSITION OF TEST SAMPLES AND EXPERIMENTAL CONDITIONS WERE THE SAME AS IN AMINOPHERASE ACTIVITY DETERMINATIONS ACCORDING TO LENARD–STRAUB[6]. ENZYME PREPARED JAN. 4, 1946.

Date of experiment	Initial amount of pyruvate or α-keto-glutarate	Disappearance or formation of pyruvate in 15 minutes (μ-mol.)			
		Activity of untreated enzyme	Residual activity of tested apo-enzyme	Additional activity with boiled tissue extract	Reactivation %
Split by acidification (*p*H 2·8)					
	Pyruvate				
8·1	300	78	0	26	33
16·1	307	101	0	28	27
19·1	388	129	0	44	34
23·1	311	117	0	81	70
	α-keto-glutarate				
25·1	259	98	0	26	26
29·1	269	196	0	75	38
Split by alkalization (*p*H 10–11)					
16·111	320	113	85	33	28
18·111	,,	88	74	32	43
,,	,,	88	74	17	20

In some experiments prolonged dialysis against distilled water or buffer solutions of different *p*H also resulted in partially reversible inactivation of the enzyme.

Negative results were obtained in attempts to reactivate apo-glutamic aminopherase by the addition of co-aspartic aminopherase concentrate, co-decarboxylase or phosphopyridoxal[4], and by flavine-adenine-dinucleotide or thiamine.

The nature of the active group of glutamic aminopherase is under investigation.

Addendum by cable received May 25. We have reported above on the reversible splitting of glutamic aminopherase prepared according to Lènard–Straub, stage *B*; tissue Kochsaft reactivated the apo-enzyme. Further investigation showed that a purer preparation of this enzyme (Lènard's stage *D*) is split more readily upon acidification and dialysis. Reactivation can be effected by addition of Kochsaft or of 1–5 μgm. phosphopyridoxal. Larger quantities, 10–25 μgm., phosphopyridoxal fail to activate the enzyme. Our negative results with phosphopyridoxal were due to the inhibitory action of the excessive concentrations employed.

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