stantially influenced the MAO activity. No significant difference could be demonstrated between the values obtained from patients who had died after a long period of illness and from those who had died suddenly.

In the 5 cases treated with Iproniazid the values found were much lower than those observed in the controls (Figure 1). Thus in 3 cases (Nos. 3, 4, 5) treated 1–3 weeks before death with 75–125 mg daily, the MAO activity was less than 5% of the mean control value. In one case (No. 1), that had been treated with Iproniazid only on the last 4 days before death with a dose of 50 mg daily intramuscularly, the activity in the parts of the brain studied was 15% of the mean control values. The inhibition of the liver MAO in this case was still more pronounced despite the smallness of the dose of Iproniazid, the activity being less than 3% of the mean control value. This indicates that there may be a difference between the degree of MAO inhibition in different organs after treatment with MAO inhibitors. The present experiments indicate that the MAO of the liver is more accessible to Iproniazid than that of the brain. Similar observations have been made in animals[15]. It should be observed that in the case mentioned above the patient had received the Iproniazid parenterally. In another case (No. 2), which had been given Iproniazid in a dose of 50–100 mg daily for 4 weeks until 6 days before death, the activity in the brain was 15% of that in the controls, the corresponding value in the liver being about 25%. In this case, then, the inhibition was more marked in the brain, which might indicate a less rapid turnover of the brain MAO. The results from a more recent case, not included in the Figures, were in agreement with the latter observation.

The range of variation of the monoamines in the brain was somewhat wider than that of the MAO activity (Figure 2). A slow decrease of the concentration of catecholamines during the interval between death and necropsy was noted. The values observed for the DA and NA concentrations will appear from Figure 2. The mean value found for the 5-HT concentration in the hypothalamus was 0.16 μg/g. This mean was obtained from cases autopsied 10–15 h after death. The number of 5-HT determinations in the control group was not enough to assess the rate of fall, if any, between death and necropsy.

Administration of Iproniazid caused a higher monoamine level in the brain. The mean concentration of DA in the caudate nucleus and of NA and 5-HT in the hypothalamus was about twice as high in the cases which had received Iproniazid as in the controls (Figure 2). There appears to be no reason to believe that the monoamine levels observed did not reflect the concentrations present before death. The assumption is strengthened by earlier observations made in some cases that the amount of 3,4-dihydroxyphenylacetic acid, the end product after oxidative deamination of DA, was low in human brain. This indicates that no appreciable breakdown of DA by means of MAO takes place after death. It is also in agreement with the results from similar experiments made on rats in which the determinations could be made immediately after death[3]. The increase of catecholamines and of 5-HT after inhibition of MAO therefore seems to indicate that this enzyme is of importance for the metabolism of both catecholamines and 5-HT in the human brain.

The observations made in the present investigation suggest that treatment with Iproniazid in ordinary therapeutic doses has an inhibitory effect on the MAO in the brain and in the liver, and that it increases the concentration of monoamines. The psychostimulating effect of monoamine oxidase inhibitors might therefore be due to an increase of brain monoamine levels[16].

Zusammenfassung. Iproniazid in therapeutischen Dosen verursacht beim Menschen eine ausgesprochene Hemmung der Gehirn-Monoaminoxidase und eine Verdoppelung des Gehalts an 3-Hydroxytyramin, Nor-adrenalin und 5-Hydroxytryptamin.

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Conversion of β-Mercaptopyruvate to 2-Mercaptoethanol by Yeast Enzymes[1]

β-Mercaptopyruvic acid arises in animal tissues by the transamination of cysteine with an α-keto acid[2]. The metabolic fate of β-mercaptopyruvate in animal tissues has been investigated extensively. The mechanism of enzymic removal of sulfur, a reaction first observed by Meister[2], has been studied in detail in our laboratory[3–7]. Although this reaction probably is the most important pathway for enzymic degradation of β-mercaptopyruvate in animal tissues, its reduction by dihydro diphosphopyridine nucleotide (DPNH) and lactic dehydrogenase to mercaptolactic acid[8,9] is sufficiently rapid to be of physiological importance. However, certain cells (e.g. yeast) do not contain appreciable amounts of the sulfur removing enzyme and pyridine nucleotide-linked lactate dehydrogenase. Alternate pathways for the degradation of β-mercapropyruvate may therefore assume greater significance. It was indeed observed that yeast extracts produce CO_2 when incubated with β-mercaptopyruvate[8].

We have pursued the problem of the enzymatic decarboxylation of β-mercaptopyruvate in order to establish the reaction sequence in a yeast enzyme system, capable of metabolizing this acid to 2-mercaptoethanol.

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The enzyme used was a carboxylase preparation of baker's yeast, purified according to SINGER[10]. Purification was carried out until the second ammonium sulfate precipitation step[10]. Measured under standard manometric assay conditions (in 0.05 *M* citrate, pH 6.0 at 30°C) 0.2 ml of our enzyme solution evolved 2.35 μMoles of CO_2 in 3 min in the presence of 200 μMoles of pyruvate, and 1 μMole of CO_2 with 200 μMoles of β-mercaptopyruvate as substrate. The velocity of decarboxylation of β-mercaptopyruvate remained about 50% of that of pyruvate for more than 30 min. The reaction went practically to completion. The enzyme preparation desulfurated mercaptopyruvate at a rate of less than 0.03 μMoles per 3 min, thus the CO_2 evolution from mercaptopyruvate is actually a measure of the direct decarboxylation of this acid, without prior conversion to pyruvate. This was further confirmed by chromatographic separation of the 2,4-dinitrophenylhydrazone of 2-mercaptoacetaldehyde[11]. The 2,4-dinitrophenylhydrazone was extracted from a deproteinized (with 5% $HClO_4$) filtrate at 4°C with ethyl-acetate, and chromatographed directly. On phosphate impregnated paper the red colored compound migrated twice as fast as the β-mercaptopyruvate dinitrophenylhydrazone. It gave a spot test for thiol groups, and could be reduced catalytically to 2-mercaptoethylamine[11].

The conversion of β-mercaptopyruvate to 2-mercaptoethanol was followed by the spectrophotometric measurement of the disappearance of added DPNH in a system containing enzyme (crude yeast extract), and β-mercaptopyruvate. Since this enzyme preparation contained ample

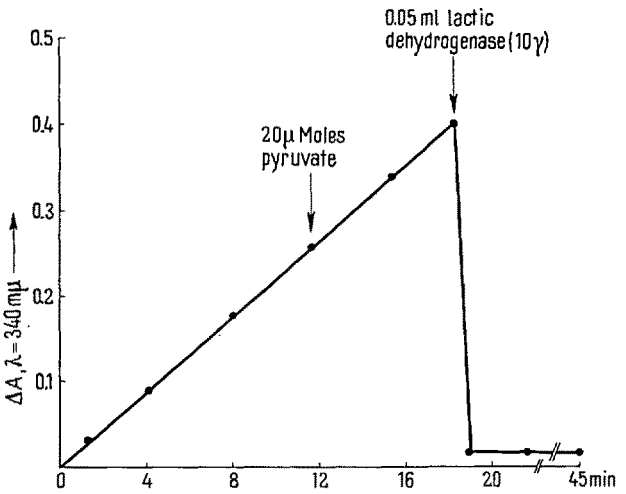

Fig. 2. DPNH formation in the presence of 2-mercaptoethanol and crystalline yeast alcohol dehydrogenase (0.1 mg) and its reoxidation by pyruvate and crystalline heart muscle lactic dehydrogenase (10 μg). The test system contained 1 mg DPN^+, 0.5 ml pyrophosphate buffer, (pH 8.5, 0.1 *M*), 0.1 ml 2-mercaptoethanol (6.5 μMoles) and H_2O to a final volume of 3.0 ml. Absorbance changes were measured at 340 mμ. Arrows indicate the time of additions of 20 μMoles of pyruvate and lactic dehydrogenase. No changes in absorbancy were observed when enzymes were omitted from the test system.

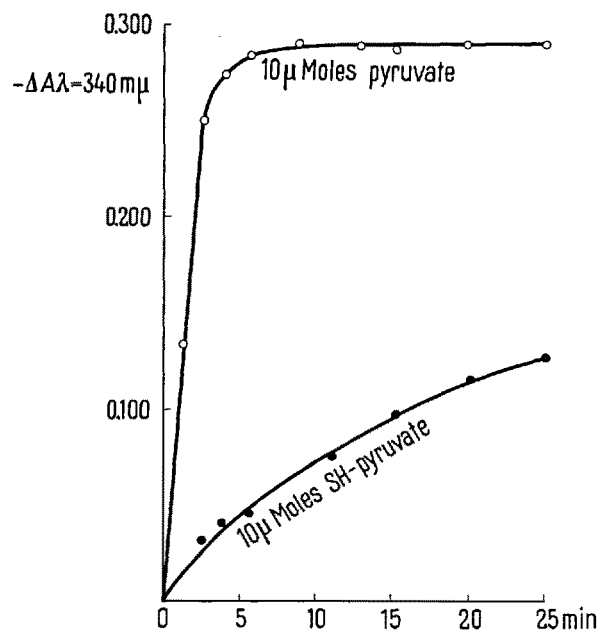

Fig. 1. Enzymatic oxidation of DPNH in the presence of yeast extract. The test system consisted of 0.5 ml yeast extract (50 g washed baker's yeast extract with 150 ml 0.06 *M* phosphate buffer, at pH 7.2, for 1.5 h, at 30°C, then diluted with 200 ml H_2O; the sediment was centrifuged at 20000 × *g* for 0.5 h at 0°C; protein content of the extract was 21 mg/ml), 3 mg DPNH, 0.3 ml tris (hydroxymethyl) amino methane buffer of pH 7.4, 0.2 *M*, and substrates plus water to make a final volume of 3.0 ml. Absorbance changes were determined in the Beckman spectrophotometer; (1 cm light path, λ = 340 mμ). The decrease in absorbance (−Δ A) is plotted against time. Corrections were made for absorbance changes, observed in the absence of added substrate.

alcohol dehydrogenase, and no pyridine nucleotide-linked lactic dehydrogenase, this optical test is a measure of the reduction of acetaldehyde to ethanol (with pyruvate as substrate) or that of 2-mercaptoacetaldehyde to 2-mercaptoethanol (with β-mercaptopyruvate as substrate) (Figure 1).

2-Mercaptoethanol itself was oxidized by crystalline yeast alcohol dehydrogenase in the presence of DPN^+. Since this reaction is complicated by the possibility of addition of thiol compounds to DPN^+[12,13] the validity of the optical density change at 340 mμ as a measure of DPNH formation was tested by the addition of pyruvate and crystalline heart muscle lactic dehydrogenase. Under these conditions the DPNH formed by oxidation of 2-mercaptoethanol is instantaneously reoxidized by pyruvate, resulting in the disappearance of the absorbance at 340 mμ. As shown in Figure 2, this dismutation reaction occurs. In separate experiments we observed that the oxidation of 2-mercaptoethanol by crystalline yeast alcohol dehydrogenase and DPN^+ at pH 7.4 was accelerated by semicarbazide.

It is concluded that in yeast the decarboxylative degradation of β-mercaptopyruvate can be coupled with the reduction of the decarboxylation product to 2-mercapto-

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ethanol. Transamination of cysteine to β-mercaptopyruvic acid, a reaction hitherto not reported in yeast, could provide a link in an anacrobic reductive fermentation pathway of this amino acid to mercaptoethanol.

Zusammenfassung. Hefeextrakt decarboxyliert β-Mercaptobrenztraubensäure zu 2-Mercaptoazetaldehyd, welches von DPNH und Alkohol-Dehydrogenase zu 2-Mercaptoethanol reduziert wird. Enzymatische und chemische Messungen bestätigen den Reaktionsmechanismus.

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Thiamine Diphosphatase in Thiamine Deficiency

Thiamine diphosphatase dephosphorylates cocarboxylase which is thiamine diphosphate. In chickens rendered thiamine deficient, there is a significant increase in the activity of thiamine diphosphatase.

Figure 1 shows the values found for the rate of liberation of inorganic phosphate in μmoles/g tissue/h. The mean enzyme activity was found to be 43.0 units for the healthy tissues of control birds on a normal diet, and 60.6 for the pathological tissues of thiamine deficient birds on a specially constructed diet on which normality could be maintained by supplements of thiamine only.

These figures show a *P*-value of the difference of means at less than 0.05. The increase in activity of thiamine diphosphatase in thiamine deficient chicken brain is significant.

Figures 2 and 3 give the results of experiments on the thiaminediphosphatase content and change in weight of chickens on a basal diet with and without thiamine.

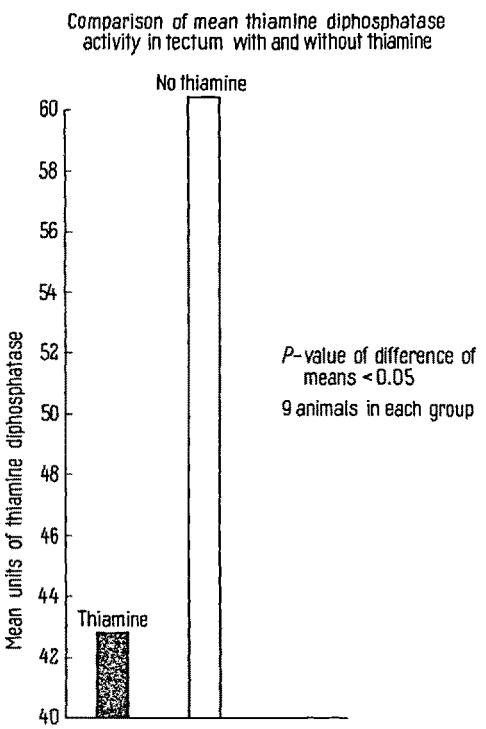

Fig. 1

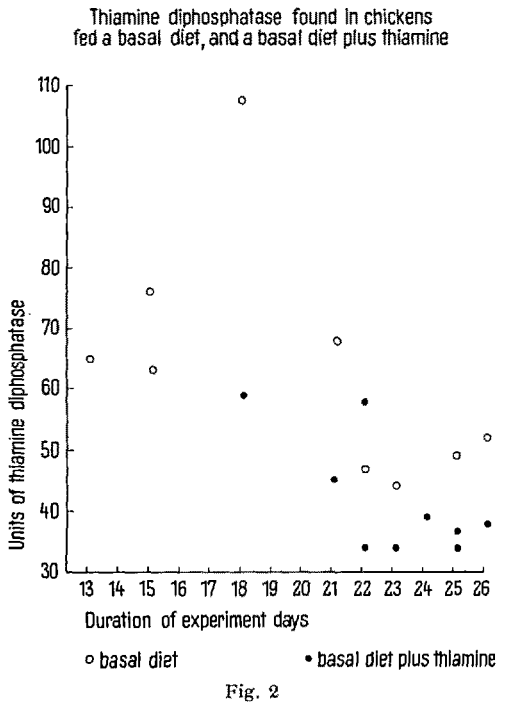

Fig. 2

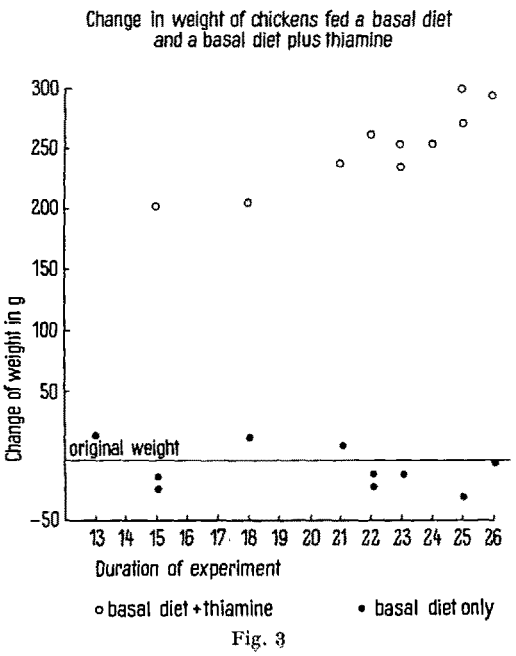

Fig. 3