sible. Thus, tryptophane and formaldehyde gave 3, 4, 5, 6-tetrahydro-4-carboline-5-carbonic acid (Found: C 66.70, H 5.26). With acetaldehyde the 3-methyl derivative was formed, which melted with decomposition at 295° (Found: C 67.66, H 5.96). Condensation with paraldol gave the 3-β-hydroxypropyl derivative melting at 261° (Found: C 65.32, H 6.57). Crotonic aldehyde gave an amorphous substance (Found: C 69.72, H 6.48), and finally with benzaldehyde the 3-phenyl derivative was obtained which melted at 223–226° (Found: C 73.85, H 5.45).

Since in the formation of these substances a new center of asymmetry is produced at carbon atom 3, the production of epimers is a possibility in all cases except that in which formaldehyde is employed. In the case of the crotonic aldehyde product, the double bond of the propenyl side chain introduces, in addition to the possibility of shift, the added complication of cis trans isomerism. The results of the examination of our substances from this standpoint will be reported elsewhere.

Finally, as a next step, attempts have been made to prepare derivatives containing an N methyl group at position 4, by direct methylation of the above substances as well as by the substitution of N-methyl tryptophane[5] for tryptophane in these condensations. Thus, benzaldehyde has given 3-phenyl-4-methyl-tetrahydro-4-carboline-5-carbonic acid, which melted with decomposition at 199–201°.

These synthetic substances, however, do not give the delicate color reaction with dimethylaminobenzaldehyde so characteristic of lysergic acid and its derivatives. In the case of the Keller reaction, only the crotonic aldehyde condensation product gives a prompt color approaching that exhibited by lysergic acid and its derivatives. The other derivatives studied, as well as harmine and harmane, give practically negative Keller reactions.

Since it is not excluded that the carboxyl group of lysergic acid may be attached to carbon atom 3 of the carboline system, parallel attempts to prepare carboline 3-carbonic acids are also in progress.

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THE PREPARATION AND DETERMINATION OF TREHALOSE IN YEAST

TREHALOSE, a non-reducing disaccharide, has long been known to occur in certain fungi. In 1925 Koch and Koch succeeded in demonstrating that trehalose is also present in yeast.[1] They extracted 40 pounds of dried baker's yeast with alcohol and obtained a crystallized product, which they identified by melting point, optical rotation and molecular weight. No information as to the yield obtained was given. Robison and Morgan,[2] using alcohol extraction and acid hydrolysis, concluded that there is about 200 mg of trehalose present per 100 grams of dried yeast.

In a study of the carbohydrates in yeast it was found that a much better yield can be obtained by a different method of extraction, so that 1 to 2 grams of crystalline trehalose can be prepared from 300 grams of yeast. Treatment of the yeast[3] with NH_2SO_4, followed by precipitation with heavy metal ($HgSO_4 + Fe_2(SO_4)_3$ in 7.5 per cent. H_2SO_4) extracts the trehalose completely, while only traces of the polysaccharides present in yeast are extracted. During the subsequent neutralization with $BaCO_3$, the small amounts of polysaccharide present are precipitated, while the trehalose remains in solution. The filtrate is freed of barium and heavy metal and concentrated in vacuo. Addition of 20 volumes of 95 per cent. alcohol precipitates some salts, which are filtered off. The solution is placed in the refrigerator. After standing overnight, or in a few days, the typical rhombic crystals of trehalose are formed, which grow considerably in size in the next ten days. It was found that during glucose fermentation the trehalose content of yeast increases markedly. Use was made of this observation in one preparation in which 300 gm of yeast were allowed to ferment 150 gm of glucose. A gram and a half of trehalose was obtained in the first crystallization and six tenths gram by working up the mother liquor. The crystals were identified by melting point, 99–99.5° (uncorrected), specific rotation, $[\alpha]_D^{22} = 185°$ (C = 0.0497), water of crystallization 9.49 per cent. (calculated = 9.5 per cent.) and preparation of the octa-acetate. This compound melted sharply at 104° (uncorrected), $[\alpha]_D^{22} = 164°$ in chloroform (c = 0.1012).

		%	%
Acetyl determination $C_{12}H_{14}O_{11}(CH_3CO)_8$	Theoretical Found	$CH_3CO = 50.73$ $CH_3CO = 50.4$	
Elementary analyses[4] Trehalose $C_{12}H_{22}O_{11}.2H_2O$ 3.980 mg yielded 2.510 mg	Theoretical H_2O and 5.54 mg of CO_2 Found	C = 38.08	H = 6.93
		C = 37.96	H = 7.06

[1] Koch and Koch, SCIENCE, 61: 570, 1925.

[2] Robison and Morgan, *Biochem. Jour.*, 22: 1277, 1928.

[3] We are indebted to Anheuser-Busch, Inc., for the supply of starch-free baker's yeast.

[4] We are indebted to Dr. Sidney Thayer, of the Department of Biochemistry, St. Louis University, for the carbon and hydrogen determinations.

[5] T. Hoshino, *Chem. Abstr.*, 29: 6596, 1935.

4.205 mg yielded 2.710 mg	H_2O and 5.830 mg CO_2		
	Found	C = 37.85	H = 7.21
Octa-acetyl trehalose $C_{28}H_{38}O_{19}$	Theoretical	C = 49.54	H = 5.65
3.440 mg yielded 1.695 mg	H_2O and 6.305 mg CO_2		
	Found	C = 49.99	H = 5.51
4.310 mg yielded 2.23 mg	H_2O and 7.83 mg CO_2		
	Found	C = 49.55	H = 5.79

Trehalose may be determined quantitatively in one gm of yeast by adding 10 cc of NH_2SO_4, diluting with water and precipitating with heavy metal. The filtrate obtained does not reduce alkaline copper solution, provided glucose added prior to the extraction of the yeast has been allowed to ferment completely. The filtrate is made normal with H_2SO_4 and hydrolyzed for 8 hours in the water bath. This converts 95 to 98 per cent. of the trehalose present to glucose. After removal of the H_2SO_4 with barium carbonate the glucose content is determined by means of the Shaffer-Hartmann copper reagent.

Fresh baker's yeast contains from 0.5 to 1.5 grams trehalose per 100 grams moist weight, the amount depending on the medium on which the yeast was grown. Aeration of a yeast suspension without added substrate lowers the trehalose content markedly. During fermentation of glucose the trehalose content may increase to 2 to 3 per cent. The biological significance of trehalose in yeast will be dealt with in a later report.

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THE ASCORBIC ACID CONTENT OF CERTAIN ORGANS OF CHICKS RAISED ON VITAMIN C DEFICIENT RATION

THE presence of a high concentration of vitamin C in the livers of chickens fed on a scorbutic diet has been reported by Hart, Steenbock and associates[1] and Carrick and Hauge[2] by biological tests. Recently Ray,[3] using the titrimetric dye method of Birch *et al.*,[4] found that although egg yolk and egg white both were devoid of vitamin C, after 4 days of incubation the embryo began to show the presence of ascorbic acid. The livers of the embryos after 15 to 19 days of incubation were found to contain 0.105 to 0.178 mg of ascorbic acid in the whole liver and after 21 to 24 days 0.226 to 0.273 per liver.

Using the titrimetric dye method, we have recently estimated the vitamin C content of different organs of over 20 chicks, fed on a diet free from vitamin C and with or without ultra-violet irradiation, from experiments on certain vitamin D studies and found that the adrenals, intestine and intestinal mucus as well as the liver all possess a high content of ascorbic acid, whether the chicks received ultra-violet irradiation or not, and further that the concentration of ascorbic acid in these organs did not vary during the growth period between the second and the third month.

Pancreas and kidney both contained a moderate amount of vitamin C, being about one third of that of the liver or intestine. The muscle was devoid of ascorbic acid. Both the intestinal contents of the small and of the large intestine possessed a trace of ascorbic acid, indicating that part of the ascorbic acid was excreted through the intestinal wall to the lumen.

Table I shows the average result obtained with the two groups.

TABLE I
ASCORBIC ACID CONTENT mg PER gm OF TISSUE

Ultra-violet irradiation	Muscle	Adrenals	Liver	Intestine	Intestinal mucus	Small intestinal content	Large intestinal content	Pancreas	Kidney
Yes	0	0.811	0.335	0.380	0.375	0.052	0.045	0.128	.100
No	0	0.915	0.302	0.391	0.404	0.047	0.056	0.134	.120

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

APPARATUS FOR THE STUDY OF SENSORY DISCRIMINATION IN MAMMALS

IN the investigation of the functional characteristics of the receptors and nervous system of animals the need for methods permitting the elicitation and objective recording of responses to external stimulation is recognized. The present paper describes such a method, which has been found to be well suited to the analysis of all phases of the visually controlled behavior of the cat, and which may be modified for the investigation of the responses to other forms of exteroceptive stimulation in various typical laboratory mam-

[1] C. W. Carrick and S. M. Hauge, *Jour. Biol. Chem.*, 63: 115, 1925.

[2] E. B. Hart, H. Steenbock, S. Lepkovsky and J. G. Halpin, *Jour. Biol. Chem.*, 66: 813, 1925.

[3] S. N. Ray, *Biochem. Jour.*, 28: 189, 1934.

[4] T. W. Birch, L. J. Harris and S. N. Ray, *Biochem. Jour.*, 27: 590, 1933.