tion in the monkey. Probably only the most highly susceptible animals respond to the weak strains when large doses are administered.

The statement has been made that the Rockefeller Institute passage "mixed virus" has gained in virulence for monkeys and declined in activity for human beings.[5] There is no experimental evidence supporting this statement. Indeed, aside from the unexplained and temporary fluctuations in activity, referred to above, this strain has remained remarkably constant; probably for this reason it has been sought by investigators in all parts of the world. The assumption that adaptation of the virus to monkeys is accomplished at the expense of diminution of pathogenic power for man is not only to go beyond existing knowledge, but is negatived by the observed effects when original human virus is employed directly for the immunization of monkeys.[6]

The term "human virus" simply means that portions of the spinal cord from fatal cases of poliomyelitis are used for the inoculation of monkeys, without having been passed through monkeys previously. This material can be preserved in glycerol, as can the monkey passage virus. When the human virus is injected successively into the skin of *Macacus* monkeys, it produces active immunity in the greater number, but paralysis in a proportion of the inoculated animals, just as the passage strains do. The same is true of early passage strains which have not had time to become specially adapted to the monkey.

Now, the human virus must have passed an indeterminate number of times through human beings in the course of the epidemics of poliomyelitis which have occurred as natural phenomena for scores of years. And yet, this virus has retained the property of both immunizing some monkeys and paralyzing others on repeated small injections. Probably the more refractory become immune, and the more susceptible paralyzed; no way is known of identifying the two varieties of animals prior to inoculation, just as there is no known method of selecting the small proportion of children falling victims to poliomyelitis during epidemics, from the large proportion passing through the outbreaks unattacked.

The experimental studies on which the statement of the effects of human strains and early monkey passage strains of the virus is based were carried out during the 1931 epidemic of poliomyelitis in New York State.[7]

[5] J. A. Kolmer and A. M. Rule, *Am. Jour. Med. Sci.*, 188: 510, 1934; J. A. Kolmer, G. F. Klugh, Jr., and A. M. Rule, *Jour. Am. Med. Assn.*, 104: 456, 1935; J. A. Kolmer, *Ann. Institut Pasteur*, 55: 365, 1935.

[6] S. Flexner, *Jour. Am. Med. Assn.*, 99: 1244, 1932.

[7] The data relating to this subject will appear in a paper to be published in the *Journal of Experimental Medicine*.

The observations on which the preceding statement is based may be summed up as follows:

(1) No adequate evidence has been presented showing that through the action of physical and chemical agents the virus of poliomyelitis may be attenuated so as to preserve its immunizing properties, while being deprived of its potential paralyzing power.

(2) The available evidence indicates that virus exposed to injurious physical and chemical agents is either inactivated (destroyed) or merely reduced in concentration. When the virus is actually destroyed, it no longer possesses immunizing power; when it is reduced in concentration, it immunizes certain animals and may paralyze others. The proof that the treated active virus has not been attenuated is provided by the recovery of fully active virus from the paralyzed animals.

(3) No evidence exists showing that passage of virus through monkeys removes its power to infect and produce paralysis in man. On the contrary, we possess convincing observations which show that an indeterminate number of passages of virus through human beings does not deprive it of its potential paralyzing effect when injected into monkeys.

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THE ERGOT ALKALOIDS. SYNTHESIS OF 4-CARBOLINE CARBONIC ACIDS

A PROVISIONAL structure for lysergic acid (3-propenyl-4-methyl-3, 4-dihydro-4-carboline-5-carbonic acid) has been suggested,[1] based on the interpretation of analytical data, of its properties and of the substances obtained from it by alkali fusion. Such a structure, if correct, would therefore place this acid in close relationship to the *harmala* group of alkaloids. Parallel with the continuation of the study of its degradation by different procedures, we have been attempting to check the validity of such a structure by synthesis.

The first steps in the synthesis have been readily realized by an extension of the method used by Tatsui[2] and by Akabori and Saito[3] in which tetrahydroharman was produced by the condensation of tryptamine with acetaldehyde. By the substitution of tryptophane[4] itself for tryptamine in this reaction we have found that a number of 3-substituted tetrahydrocarboline-5-carbonic acids have become readily acces-

[1] W. A. Jacobs and L. C. Craig, *Jour. Biol. Chem.*, 111: 455, 1935.

[2] G. Tatsui, *Chem. Centralbl.*, II: 668, 1928.

[3] S. Akabori and K. Saito, *Ber. chem. Ges.*, 63: 2245, 1930.

[4] W. O. Kermack, W. H. Perkin, Jr., and R. Robinson, *Jour. Chem. Soc.*, 119: 1616, 1921.

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.