peptone. This might suggest that other parts of amino acids beside the carboxyl and amino groups play a part in ring or chain formation, such as the phenolic group of tyrosine. We have shown that the phenolic group in tyrosine reacts with sodium in liquid ammonia to the extent of 25% of the number of groups present.

It is interesting to note that there is first a fall and then a rise in the total amount of hydrogen liberated, with a final value approaching the theoretical value for complete hydrolysis. Comparison of the curves for Armour's, Merck's, and Witte's peptone suggest that the difference between the curves may be due to the extent of hydrolysis, in addition to the type of protein used. We think of two possible explanations for the differences noted in the properties of the silk peptones. It is possible that with decreasing length of the peptide chain, there is an increased ease with which the imide group may be reduced. The other possible explanation is that as hydrolysis of the silk proceeds, either rings are formed or are liberated in such a way that they become more reactive for awhile, which is followed by the hydrolysis of the rings to give peptides and amino acids progressively.

While we think that our investigations indicate the presence of rings in the proteins and peptones that we have studied, we wish to obtain more data before forming conclusions.

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Effect of Tyrosine on Botulinum Toxin.

WILLIAM BURROWS. (Introduced by N. Paul Hudson.)

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We noted, in a study of the growth of *Clostridium botulinum* on synthetic mediums,[1] considerable irregularity of toxin production, particularly among the type B strains.

A number of possible explanations for this phenomenon were considered. One which seemed most plausible arose from the work of Kempner and Schepilewsky,[2] who, stimulated by the work of Wassermann and Takaki[3] on the detoxification of tetanus toxin with brain emulsions, reported that brain emulsions and a water-

[1] Burrows, W., *J. Inf. Dis.*, 1933, **52**, 209.

[2] Kempner and Schepilewsky, *Z. f. Hyg.*, 1898, **27**, 213.

[3] Wassermann and Takaki, *Ber. klin. Wchschr.*, 1898, **1**.

insoluble component of brain would neutralize botulinum toxin. In a search for specific chemical compounds which would have this effect, they tested tyrosine, since Phisalix[4] had reported that the amino-acid would neutralize snake venom. They presented evidence indicating that tyrosine would neutralize botulinum toxin. This neutralizing capacity was quite limited, for white mice could be protected against only 3 MLD of toxin. This protection, however, was manifested not only when tyrosine and toxin were injected simultaneously, but also when the injection of tyrosine was some 24 hours before that of toxin.

Since most of the synthetic mediums referred to above contained tyrosine to the limit of its solubility, 3 possibilities were considered: (1) these quantities of tyrosine interfered with the formation of toxin; (2) the tyrosine present had a deleterious effect on formed toxin; and, (3) tyrosine injected into mice incidental to the injection of toxin protected the animals in some unknown way. We therefore tested these possibilities experimentally. We have used phenylalanine in addition to tyrosine because of the close structural relationship of the two.

The organisms were grown in beef heart medium saturated with tyrosine and in similar mediums containing phenylalanine in the same amount, *i. e.,* 40 mg. per 100 cc. of medium. We have also used mediums containing both amino-acids. In only 2 experiments in a series of 10 were there significant differences in toxin titrations between the tyrosine-containing mediums and control mediums to which tyrosine had not been added. These lowered titres were found only in type B cultures in the tyrosine mediums. Phenylalanine produced no effect whatever.

Other experiments were carried out in which the protection tests of Kempner and Schepilewsky were repeated. We have also incubated mixtures of toxin and tyrosine for periods ranging from 30 minutes to 2 hours. These experiments indicated quite definitely that injections of tyrosine, either simultaneous with the injection of toxin or 24 hours previously, would not protect mice against 3 MLD of toxin. Tyrosine was injected in quantities of 50 to 100 mg. It was also apparent from these experiments that incubation of saturated tyrosine solutions for these periods of time with toxin had no deleterious effect on the toxin itself.

Coleman,[5] in a study of peptone therapy, repeated the work of

[4] Phisalix, *Compt. Rend.*, 1898, p. 413 (quoted by Kempner and Schepilewsky).

[5] Coleman, *J. Inf. Dis.*, 1924, **34**, 614.

Kempner and Schepilewsky on brain emulsions and was unable to confirm their results.

Summary. We have failed to protect mice against small amounts of botulinum toxin by the injection of tyrosine and have not been able to confirm the work of Kempner and Schepilewsky. We feel that the suggestive results of 2 of our experiments on the effect of excess quantities of tyrosine on the formation of botulinum toxin are not significant in the light of the other experiments described here. We have also found that neither tyrosine nor phenylalanine had a deleterious effect on formed toxin when mixtures of the amino-acids and the toxic material were incubated together.

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Experimental Production of Gastric and Duodenal Ulcers in Dogs in Cinchophen Poisoning.

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Churchill and Van Wagoner[1] attempted to produce acute yellow atrophy of the liver by feeding cinchophen to dogs. Functional and histological damage was produced with large doses, but this did not simulate acute yellow atrophy. Numerous acute gastric and duodenal ulcers were observed at autopsy. Smaller doses of cinchophen gave definite functional damage of the liver, but histological damage was irregularly produced.

A total of 25 dogs were given cinchophen in varying doses from 22 mg. per kilo to 27 times that amount. In this series acute and chronic gastric ulcers were found in over 80% of the dogs used. These ulcers were typical grossly and microscopically. These results were published and the following possibilities of the mechanism of the production of these ulcers were listed[2]: 1. Cinchophen may have a direct toxic action on the gastric mucosa, combined with digestion by the gastric secretions. 2. The drug may combine with the mucin of the stomach and thus remove the normal protection of the gastric mucosa. 3. It may act on the autonomic nervous system and induce erosions by its neurogenic effect. 4. Cinchophen may

[1] Churchill and Van Wagoner, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 581.

[2] Churchill and Van Wagoner, *Arch. Path.*, 1932, **14**, 860.