PROCEEDINGS

OF THE

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

VOL. 30. APRIL, 1933. No. 7.

Illinois Section.

Northwestern University Medical School, March 14, 1933.

6690

Reactions of Peptones with Metallic Sodium in Liquid Ammonia.

R. G. ROBERTS AND C. O. MILLER. (Introduced by C. J. Farmer.)

From the Northwestern University Medical School.

We have previously reported[1] that certain proteins, amino acids, dipeptides, and diketopiperazine are acidic in liquid ammonia, and that they react with metallic sodium to liberate hydrogen with the formation of the corresponding ammono salt. We have also studied the action of sodium upon peptones under similar conditions. The following peptones were used: Armour's Meat Peptone, Merck's Meat Peptone, Witte's Peptone, and silk peptones prepared by treating silk fibroin with 70% sulfuric acid at room temperature for one, 2, 3, 4, and 10 days. We were interested in comparing the properties of peptones in liquid ammonia with the properties of proteins, amino acids, dipeptides, and diketopiperazines, and in comparing the properties of the various peptones with each other.

The procedure was essentially the same as described previously. Peptones are somewhat soluble or are dispersed in liquid ammo-

[1] Miller, C. O., and Roberts, R. G., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 535.

nia. The solubility of silk peptones varies with the extent of digestion, passing through a maximum on the third day.

In general, we have found that the peptones, Armour's, Merck's, Witte's, and 2 and 3 day silk peptones behave more like diketopiperazine than like proteins, amino acids, or peptides. When the quantity of hydrogen liberated by a definite amount of dried peptone is plotted against the amount of sodium that is brought into reaction, the curves for these peptones resemble closely the curve for diketopiperazine. With small amounts of sodium, relatively smaller quantities of hydrogen are liberated and greater proportion of the hydrogen is taken up by the peptone. With increasing amounts of sodium, there is a relative increase in the quantity of hydrogen liberated. The maximum is reached when the proportions of 4 gm. atoms of nitrogen are treated with 7.3 gm. atoms of sodium with the liberation of 1.2 gm. atoms of hydrogen. If peptones contained only free carboxyl groups, free amino groups and acidic imide groups, and if there was no reduction taking place, as is the case with a dipeptide, then for each 4 gm. atoms of nitrogen present in imide combination, 4 gm. atoms of hydrogen should be liberated. Since the number of carboxyl groups is approximately equal to the number of amino groups, then the N:Na:H should be 1:1:1. We did not find the imide group of dipeptides to be acidic although the imide group of N-methyl acetamide liberates 1 gm. atom of hydrogen. It is possible that the imides of diketopiperazines are acidic in the lactam form or through tautomerization to the lactim form.

In the case of the silk peptones which were hydrolyzed by 70% sulfuric acid at room temperature for 1, 2, 3, 4, and 10 days, there is a marked difference in the maximum quantity of hydrogen which is liberated when they are treated with an excess of sodium. Calculated for 4 gm. atoms of nitrogen, native silk gives 2.70 gm. atoms of hydrogen, 1 day peptone 2.35, 2 day peptone 1.07, 3 day peptone 1.15, 4 day peptone 2.63, and 10 day peptone 2.87. It is interesting to note that the change in solubility or dispersion with length of digestion period parallels this curve. Qualitative observations show that the rate at which hydrogen is liberated increases during the first 3 days, but remains constant thereafter. Furthermore, when glycine, tyrosine, and alanine are mixed in the proportions in which they are present in silk and treated with excess sodium in liquid ammonia, they do not liberate 4 gm. atoms of hydrogen for 4 gm. atoms of nitrogen, but only 2.86 gm. atoms of hydrogen. This is the same quantity that was liberated by the 10 day

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.

6691

Effect of Tyrosine on Botulinum Toxin.

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

From the Department of Hygiene and Bacteriology of the University of Chicago.

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**.