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Some Reactions of Ammonolyzed Epinephrine.

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(Introduced by William S. Hoffman.)

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Previous work[1] on certain nitrogenous substances in liquid ammonia has shown that some of them tend to ammonolyze with changes in their physical, chemical, and biological properties. Ammonolyzed edestin was reported[2] not to have given an anaphylactic reaction with a guinea pig previously sensitized to the untreated edestin. Recent observations on changes in some physical constants of certain amino acids in liquid ammonia indicate an ammonolysis. Epstein, Gunn and Virden[3] have shown changes brought about in the physiological activity of phenylethylamines by methylation. These facts, in addition to the observations of Kraus and White[4] that the hydroxyl groups of alcohols and phenols are quite reactive in liquid ammonia and to the report of Roberts and Miller[5] that the phenolic group of tyrosine seems to be acidic in liquid ammonia have led us to believe that epinephrine could be ammonolyzed and thus altered in its physiological activity.

The chemical treatment consisted of adding 60 mg. of Parke, Davis adrenalin to 150 cc. of liquid ammonia contained in a Dewar flask connected by gas-tight stoppers and tubing to a mercury seal allowing the ammonia to vaporize without admitting moisture to the adrenalin. The liquid ammonia, previously dried over sodium by the method of Fernelius and Johnson, boils off in about 24 hours, and the drug is ready for use. The yellowish adrenalin base darkens to a reddish-brown on standing in liquid ammonia, and its solubility in water and in glycerine decreases.

The flaky material is powdered, and used in the form of an aqueous sol. With glycerine used as a vehicle, the suspension is more uniform, and the base has less tendency to settle out. When water is used, the injection is made at once since the ammonolyzed base hydrolyzes on standing. With glycerine, a pressor activity characteristic of the new compound was obtained after 30 days, but

[1] Miller and Roberts, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 533.

[2] Private communication from E. Gebauer-Fuelnegg.

[3] Epstein, Gunn and Virden, *J. Physiol.*, 1932, **76**, 224.

[4] Kraus and White, *J. Am. Chem. Soc.*, 1923, **45**, 768.

[5] Roberts and Miller, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 821.

after standing for 6 months an adrenalin curve was again obtained on injection.

Dogs were the only animals used, and the drugs were administered by injection into the femoral vein. Either pentobarbital or ether were used as anesthetics, and water or glycerine were the only vehicles. When the ammonolyzed adrenalin, usually in dilution of 1:20,000, was injected, a curve was obtained that in a general way resembled the curve obtained from the untreated adrenalin control, but the initial rises were less rapid, the peak of the secondary curve was more sustained and lengthened giving an increased time above the base line of about 50% in general and the heart beat was markedly increased. The compound's activity is decreased after reduction with sodium.

Alles[6] in studying the pressor and toxic effects of phenylethyl and phenylisoprophylamine decided that the side chains did not affect the intensity factor, but did affect the duration factor although the introduction of hydroxyl groups into the phenyl group also played a part in duration as well as in intensity.

In comparing the chemical structure and pressor activity of known pressor substances, such as epinephrine, synephrine, tyramine and ephedrine, it would seem that blocking the adjoining catechol hydroxyl groups of epinephrine by methylation or ammonolysis would prolong its pressor effect. We observed no toxic effect from ammonolysis as has been reported from methylation. It seems most likely to us that the hydroxyl groups of the phenyl radical have been converted into ammonia addition products rather than into amines and, possibly the hydroxyl of the side chain and the trivalent nitrogen have been affected. Possibly treatment in liquid methylamine would give results that would be more satisfactory than either methylation or ammonolysis.

We plan to make a study of the nitrogen distribution in the ammonolyzed adrenalin, and to complete other work that we have done on the ammonolysis of ephedrine, synephrine, atropine, pilocarpine, ergotamine, and insulin.

[6] Alles, *J. Pharm. and Exp. Therap.*, 1933, **47**, 351.