purine in other biological systems has been reported(22-23), studies of such possibility in the case of kappa were made. Concentrations of test purine in 0, 5, 10, 25, 50, 100, 250, and 500 μg/ml were used in a mixture with 500 μg/ml of 2,6-diaminopurine. After a 24-hour exposure, isolations and killer-sensitive tests were run as previously described. Adenine, adenosine, and adenylic acid were found to block the effect of 2,6-diaminopurine on kappa(21).

Additional evidence is being sought through staining procedures, electron microscopy, and radioautography on the question of whether the amount of effective kappa is reduced by 2,6-diaminopurine through prevention of its formation, through production of an abnormal kappa, or by its actual destruction.

Summary. The content of effective kappa in *Paramecium aurelia* killers is markedly reduced by their exposure to 2,6-diaminopurine. This purine uniquely exhibits this capacity among the many purines and pyrimidines tested. The effect is blocked by the simultaneous presence of adenine, adenosine, or adenylic acid.

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Detection of Norepinephrine in the Parotid Gland Secretion of *Bufo agua*.* (19249)

LOUIS LASAGNA.† (Introduced by E. K. Marshall, Jr.)

From the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore.

The presence of epinephrine in the parotid gland secretions of various species of toads has

* This species of toad is known by various names. *Bufo agua* and *Bufo marinus* have been most frequently used. The "glands" referred to are aggregates of glands behind each ear, and are usually designated as either "parotid" or "parotoid". The term parotid is used here in its literal sense since in structure, function, and content the toad gland described above is completely different from the salivary gland which is commonly associated with the term.

been known for many years(1-5). Recent investigations have suggested the frequent coexistence of norepinephrine and epinephrine in animal tissues, with detectable amounts of both having been reported in the adrenal gland, chromaffine tumors, sympathetic nerves, and most organs(6). Bacq and LeComte, however, found no trace of norepinephrine in the secretion of *Bufo arenarum* (7). Fischer and LeComte, working with *Bufo marinus,* could detect norepinephrine in but one parotid gland out of 54 studied, but noted that in each assay on the cocaine-sensitized nictitating membrane, the response was greater than that expected from the epinephrine content as determined by a fluorometric method(8). Recently, Lee and Chen have reported the presence of small quantities of norepinephrine in an extract of Chinese toad venom derived from the parotid secretion of *Bufo bufo gargarizans,* using chromatography both for detection and for preparation of material for subsequent bioassay(9).

The work to be described is the result of attempts to determine the presence of norepinephrine in a preparation of epinephrine derived from the parotid glands of the giant tropical toad *Bufo agua.* The epinephrine had been prepared by Abel in 1914, presumably according to the method described in the paper of Abel and Macht(1). It had been kept in this laboratory in a tightly-stoppered weighing bottle since the time of its preparation, and was in the form of a brown powder. Utilizing the modified Folin method of Barker, Eastland, and Evers(10), 1 μg of the toad "epinephrine" was found to give the same color intensity as 0.93 μg of pure epinephrine.‡ Since pure norepinephrine produces less intense color, mg per mg, than epinephrine, its presence in the extract would make the percentage of catechols higher than that indicated by the above figures.

Paper chromatography. The technic utilized was essentially that of James(11). Epinephrine, norepinephrine, and the material under investigation were dissolved in acid alcohol and applied to Whatman No. 1 filter paper along a line 1½ inches from the bottom of the sheet. A cylinder was then formed by approximating two edges with cellulose tape as described by Crawford and Outschoorn (12). The paper cylinder was then set in a tall glass jar containing a layer of phenol saturated with water at its bottom, the top of the jar covered to maintain saturation of the atmosphere, and the phenol allowed to ascend by capillary attraction for 16-20 hours. At the end of this time, the paper was removed, dried at room temperature, and developed with 0.44% potassium ferricyanide in a phosphate buffer of pH 7.8. The epinephrine standard was found to have an R_F of 0.49 to 0.58 and to yield a pink-red color which faded to a yellow-brown. The norepinephrine standard had an R_F of 0.27 to 0.33, and produced a lavender spot which faded to a chocolate-brown. The toad "epinephrine" yielded two distinct spots—a large one with an R_F of 0.52 to 0.56 and a much smaller one with an R_F of 0.32 to 0.33. Not only did the spots correspond in location to those produced by the pure standards, but their colors were identical. Quantitative assay was attempted by a comparison of the sizes of spots produced by varying mixtures of epinephrine and norepinephrine with those produced by samples of the toad "epinephrine." By this crude bracketing technic, the "epinephrine" was found to contain between 2 and 5% of norepinephrine (as base).

Chemical determinations. The Auerbach and Angell method(13) was employed with one modification. It was found that identical amounts of norepinephrine produced different intensities of color, depending on the amount of epinephrine present in the sample. Therefore, 3 standard curves were run, determining the color intensity produced by 0.05 to 0.2 mg norepinephrine in the presence of 2 and 4 mg

† Sharp and Dohme Fellow in Pharmacology and Experimental Therapeutics.

‡ Synthetic l-norepinephrine and l-epinephrine, in the form of the bitartrates, were used as standards throughout this work. The l-norepinephrine contained less than 0.5% of d-norepinephrine. The l-epinephrine contained less than 0.1% of d-epinephrine, and less than 0.1% norepinephrine. We are indebted to Dr. John C. Seed of Sterling-Winthrop Research Institute both for a generous supply of these compounds, and for the above data on their purity.

epinephrine as well as the 1 mg recommended by the original authors. Samples of the toad "epinephrine" were then run through, in amounts of 1, 2 and 4 mg total base. The readings in each instance were read against blanks run with equivalent amounts of epinephrine, and the readings translated into the amount of norepinephrine present by use of the appropriate standard curve. The results obtained in this manner checked very well, with the percentage of norepinephrine ranging from 4.9 to 5.7%.

Discussion. The chromatographs clearly indicate the presence of at least two separate components in the toad "epinephrine" of Abel and Macht. These components migrate at rates identical with those of epinephrine and norepinephrine, and yield the same color reactions. In addition, the chemical studies disclose the presence of a compound which behaves like norepinephrine. The percentages of norepinephrine obtained with these two different technics are in good agreement. Further, Lee and Chen, using a similar toad preparation, have obtained effects on the blood pressure and nictitating membrane of the cat, with material eluted from the "norepinephrine area" of large scale paper chromatographs, that duplicate those produced by pure l-norepinephrine. Their estimate of 1-2% norepinephrine content is of the same order of magnitude as our figures. These data complement each other and strongly suggest that the second component is, indeed, norepinephrine.

It is of interest that Fischer and LeComte detected 0.5 μg norepinephrine per 10 μg of epinephrine in one of their gland preparations, and that their results with the cocaine-sensitized nictitating membrane were compatible with the presence of small amounts of norepinephrine in all of their extracts. These somewhat equivocal findings and the inability of Bacq and LeComte to demonstrate norepinephrine are easily explained by the small quantities of norepinephrine which seem to be present in toad secretions. Neither bioassay nor most of the currently available chemical technics could be expected to reveal clearly the presence of percentages of norepinephrine of 5% or less of the catechol content. As Lee and Chen point out, these problems are magnified by the use of crude secretion. It is suggested that in investigations of the epinephrine-norepinephrine content of biological material chromatographic technics should be considered if cruder methods do not reveal the presence of both amines.

The existence of norepinephrine in toad secretions demonstrates the ability of these animals to form the amine. This raises the possibility that other tissues of the toad, including those of the nervous system, may possess this ability, and that norepinephrine may play an important role in the normal physiology of these animals, perhaps analogous to that suggested for higher animals(14).

Summary. Chemical and chromatographic studies have revealed the presence of a substance, possessing characteristics of norepinephrine, in a preparation of "epinephrine" extracted from the parotid secretion of *Bufo agua*. This substance was present in amounts of 2-5%.

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