1. Hertz, R., and Sebrell, W. H., *Science,* 1944, v100, 293.
2. Goldsmith, E. D., Schreiber, S. S., and Nigrelli, R. F., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 299.
3. Hertz, R., and Tullner, Wm. W., *Endocrinol.,* 1949, v44, 278.
4. Hertz, R., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 113.
5. Zarrow, M. X., Hisaw, F. L., and Salhanick, H. A., *Science,* 1950, v112, 147.
6. Du Bois, K. P., Cochran, K. W., and Mazur, M., *Science,* 1949, v110, 420.
7. Ross, M. H. and Ely, J. O., *J. Cell. and Comp. Physiol.,* 1949, v34, 71.
8. Koch, F. C. and McMeekin, T. L., *J. Am. Chem. Soc.,* 1924, v46, 2066.
9. Baker, J. R., *Quart. J. Micr. Sci.,* 1944, v85, 1.
10. Bensley, C. M., *Stain Tech.,* 1939, v14, 47.
11. Dietlein, L. F., 1951, unpublished data.
12. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.,* 1933, v100, 485.

Received May 3, 1951. P.S.E.B.M., 1951, v78.

An Influence of 2,6-Diaminopurine upon the Content of Kappa in *Paramecium aurelia,* Variety 4.* (19248)

C. CHESTER STOCK, WINIFRED JACOBSON, AND MARY WILLIAMSON.

From the Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.

Many compounds which, from their structure, might be expected to interfere with nucleic acid metabolism, have been tested for their ability to restrain the growth of neoplasms in experimental animals(1-7). The studies are based upon an assumption that the metabolism, the pattern of uptake of precursors of nucleic acid in normal cells, is different from that in neoplastic ones. It is assumed, furthermore, that the differences are sufficiently important to permit their use in inducing by anti-metabolites the selective injury of the neoplastic cell type, as compared with the normal. This approach to cancer chemotherapy has the advantage, in theory, of being applicable whether the neoplastic process be due to a virus or to a mutated genic structure, since both are dependent for function on their component nucleic acids. Of the compounds which have been synthesized and tested as potential antimetabolites of nucleic acid, few have exerted a high degree of growth restraint of any type of neoplasm *in vivo.* None have been curative. Nevertheless, *in vitro,* a substantial degree of selective injury to some, but not all, neoplastic cells, as compared with normal types growing at the same rate and of generally similar origin, has been demonstrable (8).

The possibility of differential toxicity based upon the presumed specificity of nucleic acid metabolism of different type cells is attractive and important. Accordingly, new means were sought to provide evidence pertinent to this assumption. The characteristics of the cytoplasmic factor kappa in the ciliated protozoan, *Paramecium aurelia,* as elucidated by Sonneborn and his associates(9-18), indicated that it might provide a useful subject for study. Certain stocks of *P. aurelia,* known as killers, contain a sufficient concentration of kappa particles to produce paramecin, a substance lethal to other stocks of the protozoan. The susceptible animals, known as sensitives, contain no kappa particles and form no paramecin. X-ray inactivation studies and staining procedures indicate that the kappa particle is roughly comparable in size to certain viruses and rickettsia. Kappa particles are self-duplicating at rates independent of the fission rate of the paramecia containing them, and contain desoxyribonucleoprotein(15,16), as does their product, paramecin(17). Could kappa, an easily demonstrable and functionally unique cell constituent, be destroyed with-

* We wish to acknowledge support of this study by funds from the American Cancer Society.

out injury to its host, strong evidence for a high degree of spccificity of the nucleic acid anti-metabolite would be at hand. This would lend support to further studies of compounds with similar action for their chemotherapeutic value in cancer control.

Method. At 27°C, and with an abundant supply of nutrients, kappa in variety 4, stock 51 killer paramecia can duplicate at approximately the same rate as the animals containing it. At 33.8°C(11), however, kappa particles are maintained without increase in number, while paramecia continue to reproduce. Thus, kappa may be so dispersed that many organisms will contain no kappa, and as a result, not only lose their ability to produce paramecin, but also become sensitive. Kappa in animals with a partially depleted supply may be built up to a higher concentration by returning them to 27°C and reducing their reproduction to 1 fission per day by limitation of food supply. Such animals will again produce paramecin when a sufficient kappa concentration has been restored. These methods have been applied in the expansion technic(11,14,18) for quantitative determination of kappa particles present in paramecia subjected to conditions which might decrease the amount of kappa. A nitrogen mustard, HN2, (methyl bis(beta-chlorethyl)amine), has been shown to inactivate kappa(18). Compounds of that type are highly reactive and may exert their effects by combination with a number of chemical groups and at many sites in a molecule(19). Hence, their mechanism of action is difficult to ascertain. HN2 is reactive for such a limited time, furthermore, that the reported experiments with it required modification before they could serve as models for tests of materials such as potential anti-metabolites of nucleic acid.

A screening procedure utilizing the killer-sensitive test(11) was employed to detect the capacity of test substances to affect significantly the concentration of kappa and the production of paramecin. Test compounds were dissolved in a mixture of 1 part baked lettuce culture medium(11) and 3 parts salt solution(20) in concentrations of 100, 200, 500, 1000, and 1500 μg/ml at an adjusted pH of 6.4 to 7.0. Ten to twenty non-autogamous, semi-starved killers (stock 51, mating type VII, variety 4), were exposed in a 0.2 ml volume of each concentration for 24 hours at 27°C. For controls, an identical group of killers was incubated under the same conditions in a 0.2 ml volume of the culture medium-salt solution mixture. Three paramecia were then isolated from each of the two highest concentrations of the compound which the animals survived. Similar isolations were made from the control cultures. The isolated organisms were placed in fresh culture medium and allowed to reproduce at maximum rate for 48 hours, at which time they had undergone 9 or 10 fissions. Killer-sensitive tests were then made on the clones from the isolated animals; *i.e.*, they were tested for their ability to kill sensitives and also for their susceptibility to killers. A clone control without added test animals indicated whether autolethality was present. After incubation for 24 hours at 27°C, the tests were observed for lethal action. It was found that the progeny of killers exposed to 2,6-diaminopurine† in concentrations of 500 μg/ml were markedly weakened in killing ability and that some had become sensitive, as indicated by autolethality in the clone control and susceptibility to test killers. Observations at 48 hours indicated that as the animals became more starved under the test conditions, thereby decreasing their fission rate, the ability to kill was strengthened.

To obtain quantitative information on the decrease in effective kappa resulting from exposure to 2,6-diaminopurine, data were sought through the expansion technic(11). The observations indicate a decrease in paramecin-producing kappa to 5-10% of the level in untreated animals(24). Many other purines and pyrimidines, including 8-aza-adenine, 8-azaguanine, and substituted 2,6-diaminopurines were tested and none have exerted an effect similar to 2,6-diaminopurine. These experiments will be reported later(21).

Since the ability of natural purines to block or reverse the inhibitory effects of 2,6-diamino-

† We are indebted to Dr. George H. Hitchings, Wellcome Research Laboratories, Tuckahoe, N. Y., for supplying this material.

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.

1. Parsons, L. D., Gulland, J. M., and Barker, J. R., *Symp. Soc. Exp. Biol.*, 1947, v1, 179.

2. Kidder, G. W., Dewey, V. C., Parks, R. E., Jr., and Woodside, G. L., *Science*, 1949, v109, 511.

3. Stock, C. C., *Am. J. Med.*, 1950, v8, 658.

4. Gellhorn, A., Engelman, M., Shapiro, D., Graff, S., and Gillespie, H., *Cancer Research*, 1950, v10, 170.

5. Burchenal, J. H., Johnston, S. F., Burchenal, J. R., Kushida, M. N., Robinson, E., and Stock, C. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 381.

6. Elion, G. B., Buckley, S. M., Stock, C. C., and Hitchings, G. H., *Cancer Research*, 1951, v11, 246.

7. Skipper, H. E., Mitchell, J. H., and Bennett, L. L., Jr., *Cancer Research*, 1950, v10, 510.

8. Biesele, J. J., Berger, R. E., Wilson, A. Y., Hitchings, G. H., and Elion, G. B., *Cancer*, 1951, v4, 186.

9. Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, 1943, v29, 329.

10. ———, *Cold Spring Harbor Symp.*, 1946, v11, 236.

11. ———, *J. Exp. Zool.*, 1950, v113, 87.

12. ———, *Scientific American*, 1950, v183, No. 5.

13. Sonneborn, T. M., Jacobson, W., and Dippell, R., *Anat. Rec.*, 1946, v96, 18.

14. Sonneborn, T. M., Dippell, R. V., and Jacobson, W., *Genetics*, 1947, v32, 106.

15. Preer, J. R., Jr., *Genetics*, 1950, v35, 344.

16. ———, *Am. Nat.*, 1948, v82, 35.

17. Van Wagtendonk, W. J., *J. Biol. Chem.*, 1948, v173, 691.

18. Geckler, R. P., *Science*, 1949, v110, 89.

19. Philips, F. S., *J. Pharm. and Exp. Ther.*, 1950, v99, 281.

20. Taylor, C. V., and Van Wagtendonk, W. J., *Phys. Zool.*, 1941, v14, 431.

21. Williamson, M., Jacobson, W., and Stock, C. C., To be published.

22. Hitchings, G. H., Elion, G. B., Vanderwerff, H., Falco, E. A., *J. Biol. Chem.*, 1948, v174, 765.

23. Biesele, J. J., Berger, R. E., and Hitchings, G. H., *Cancer Research*, 1950, v10, 204.

24. Jacobson, W., Williamson, M., and Stock, C. C., To be published.

Received November 28, 1951. P.S.E.B.M., 1951, v78.

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.