with a high degree of certainty, that the mutagen interferes with developmental process. The difference in effect obtained with BUDR and 5-fluorouracil may be due to the different strains of mice used as well as to the nature of the compounds and methods of observation.

JOSEPH A. DIPAOLO
Cytogenetics and Cytology Unit,
National Cancer Institute,
Bethesda, Maryland 20014

References and Notes

1. E. Freese, *Molecular Genetics* (Academic Press, New York, 1963), pt. 1, pp. 207–269.
2. M. L. Eidinoff, L. Cheong, M. A. Rich, *Science* **129**, 1550 (1959).
3. C. Danforth, *Am. J. Anat.* **45**, 275 (1930).
4. H. Chase, *Genetics* **21**, 214 (1946).
5. S. Holt, *Ann. Eugen.* **12**, 220 (1945).
6. H. Nishimura and S. Takagaki, *Acta Scholae Med. Univ. Kioto* **36**, 20 (1959).
7. H. Nishimura and K. Nakoi, *Science* **127**, 877 (1958).
8. J. DiPaolo, *J. Am. Med. Assoc.* **183**, 139 (1963).
9. C. Dagg, *Am. J. Anat.* **106**, 89 (1960).
10. C. Heidelberger, N. Chaudhuri, P. Danneberg, D. Mooren, L. Grissbach, R. Duschinsky, R Schnitzer, E. Pleven, J. Scheiner, *Nature* **179**, 663 (1957).
11. L. Bosch, E. Harbers, C. Heidelberger, *Cancer Res.* **18**, 335 (1958).

3 June 1964

Histochemical Demonstration of Uptake of Exogenous Norepinephrine by Adrenergic Fibers in vitro

Abstract. *Adrenergic fibers in isolated iris preparations were demonstrated histochemically by means of the formaldehyde condensation technique. In the presence of adenosine triphosphate (ATP) there was a net uptake of norepinephrine added in vitro by the adrenergic fibers producing a striking increase in fluorescence. In the absence of ATP only a slight increase in fluorescence was observed when the tissue was exposed to high concentrations of norepinephrine.*

Several reports indicate that exogenous norepinephrine can be taken up by tissues containing adrenergic fibers (*1*); however, the site of norepinephrine concentration within the tissue is not established. Studies with tritiated norepinephrine indicate that at least part of the exogenous norepinephrine is found in the norepinephrine-containing particles within adrenergic nerve fibers (*2*). These findings suggest that exogenous norepinephrine can enter the adrenergic storage sites but do not show conclusively whether the presence of tritiated norepinephrine in the adrenergic "granules" reflects true net uptake or simple exchange diffusion. Other studies indicate a net uptake of norepinephrine by certain tissues (*3*). A decrease in the spontaneous release of norepinephrine contained within isolated adrenergic nerve "granules" in vitro has been shown by von Euler and Lishajko (*4*). This was found to be potentiated by the presence of adenosine triphosphate (ATP) in the medium.

Falck's method (*5*) for the histochemical demonstration of norepinephrine in isolated tissues of rat iris was used. This method depends on the production of fluorescent condensation products of norepinephrine with formaldehyde (*6*). The freshly removed rat iris was washed in Tyrode's solution, mounted on a slide, and allowed to dry in room air for 10 minutes. It was subsequently exposed to paraformaldehyde fumes in a closed vessel at 80°C for 35 minutes and examined under the fluorescence microscope (*7*). A mercury vapor lamp was used as the light source with a Schott BG 12 or a Corning 5-58 filter. A Kodak No. 15 filter was used at the ocular. This arrangement permits the observation of the green fluorescence of the condensation product after excitation with a light spectrum which includes mainly the 405 mμ and 436 mμ mercury lines.

Figure 1 shows an iris preparation photographed at optimum exposure for the demonstration of the fluorescent fibers. It shows the characteristic network of the terminal adrenergic plexus. When similar preparations were first exposed to norepinephrine in the presence of ATP and subsequently treated with formaldehyde, a striking increase in the intensity of the fluorescence of the fibers was observed. This increase was so marked that it was impossible to obtain clear photographs of these tissues if the same exposure and development times were used as in the controls. The photomicrographs in Figs. 2 and 3 were therefore taken at a reduced photographic exposure. Figure 2*a* shows a typical area of an iris treated with paraformaldehyde immediately after removal. The characteristic network of fluorescent fibers can be seen in this preparation. No change was observed when similar preparations were exposed to a buffered glucose-free Tyrode's solution at *p*H 7.4. Figure 2*c* shows another iris exposed to a similar solution at the same *p*H but containing 50 μg/ml of norepinephrine and subsequently

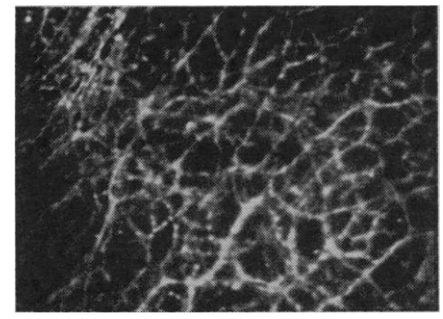

Fig. 1. Adrenergic plexus in rat iris (× 200). Polaroid No. 10,000 with 7-second exposure. Control.

washed in four changes of Tyrode's solution for 15 minutes each. For these records, identical exposure and development times were used. Only a slight increase in the fluorescence intensity of the fibers was seen in this last preparation (Fig. 2*b*) or in several other similar preparations exposed to different concentrations of norepinephrine. However, Fig. 2*b* shows that

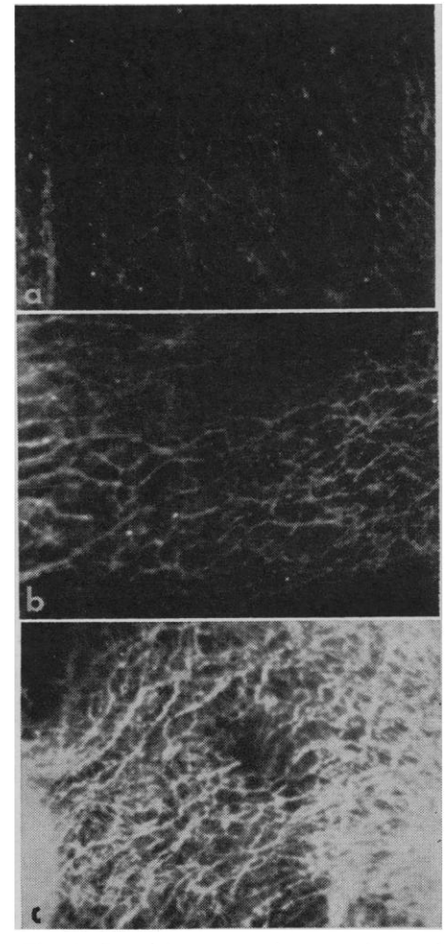

Fig. 2. Rat iris preparations at 4-second exposure. *a*, Control; *b*, in Tyrode's solution with 50 μg/ml of norepinephrine; *c*, 50 μg/ml of norepinephrine plus 1 μg/ml of ATP.

exposure of the iris tissue to the same concentration of norepinephine in Tyrode's solution in the presence of ATP (again followed by four washings in Tyrode's solution) resulted in a marked increase of the fluorescence intensity of the adrenergic fibers as well as the density of the fluorescent fibers that were visible. Even at this reduced exposure, Fig. 2*c* is too bright to show clearly the microscopic picture. The photomicrograph in Fig. 3 was therefore taken from another iris preparation treated in the same manner as in Fig. 2*c* but a reduced photographic exposure was used. The latter photomicrograph shows clearly that the uptake of norepinephrine by the tissue in the presence of ATP is confined within nerve structures.

Direct chemical determinations (*8*) of norepinephrine were made in pooled tissues from 16 irises per group. Each pool weighed approximately 9 mg. Values for the control (as in Fig. 2*a*) and thoroughly washed preparations treated with norepinephrine plus ATP (as in Figs. 2*c* and 3) were 5.2 and 36.1 μg/g, respectively.

These results indicate that in the presence of ATP, exogenous norepinephrine is taken up within the adrenergic fibers. It is of interest that in this tissue ATP is essential for a significant uptake of norepinephrine—a finding which lends support to the view that ATP is necessary for the binding of norepinephrine within the adrenergic storage sites (*9*). The results confirm previous observations made with tritiated norepinephrine and autoradiography (*10*); however, under the present conditions an extensive net uptake of norepinephrine was demonstrated.

Apart from their physiological significance, the findings indicate that this method, in which the tissue is exposed to exogenous norepinephrine in the presence of ATP, can be used in conjunction with the formaldehyde condensation procedure to provide a more sensitive histochemical method for the demonstration of adrenergic fibers in tissues. With this modification the finer branches of the fibers become visible and in general the preparations can be studied and reproduced photographically with greater ease.

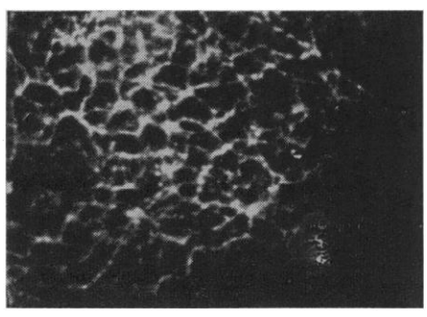

Fig. 3. A different iris preparation treated in the same way as that in Fig. 2*c* but photographed with 1-second exposure.

E. T. ANGELAKOS
Department of Physiology, Boston University School of Medicine, Boston, Massachusetts 02118

References and Notes

1. W. Raab and W. Gigee, *Circulation Res.* **3**, 553 (1955); J. Axelrod, H. Weil-Malherbe, R. Tomchick, *J. Pharmacol. Exptl. Therap.* **127**, 251 (1959); L. G. Whitby, J. Axelrod, H. Weil-Malherbe, *ibid.* **132**, 193 (1961).
2. D. E. Wolf, L. T. Potter, K. C. Richardson, J. Axelrod, *Science* **138**, 440 (1962).
3. L. L. Iversen, *Brit. J. Pharmacol.* **21**, 523 (1963); B. Bhagat, *Arch. Int. Pharmacodyn.* **146**, 47 (1963).
4. U. S. von Euler and F. Lishajko, *Acta Physiol. Scand.* **57**, 468 (1963).
5. B. Falck, *Acta Physiol. Scand. Suppl.* **197**, 1 (1952).
6. B. Falck, N. A. Hillarp, G. Thieme, A. Thorp, *J. Histochem. Cytochem.* **10**, 348 (1962).
7. American Optical model 645.
8. U. S. von Euler and F. Lishajko, *Acta Physiol. Scand.* **51**, 348 (1961); E. T. Angelakos, K. Fuxe, M. L. Torchiana, *ibid.* **59**, 184 (1963).
9. B. Falck, N. A. Hillarp, B. Hogberg, *ibid.* **36**, 360 (1956); H. Blaschko, G. V. R. Boon, A. D'Iorio, N. R. Eade, *J. Physiol.* **133**, 548 (1956).
10. T. Samorajski, B. H. Marks, E. J. Webster, *J. Pharmacol. Exptl. Therap.* **143**, 82 (1964); T. Samorajski and B. H. Marks, *J. Histochem. Cytochem.* **10**, 392 (1962).
11. With the technical assistance of Miss Mary King. The work was supported by a grant from the Council for Tobacco Research, U.S.A., and by USPHS career development award (5-K3-GM-15, 457).

20 April 1964

Hybridization of Rapidly Labeled Nuclear Ribonucleic Acids

Abstract. *RNA, prepared from strain L fibroblasts which were incubated for 30 minutes in radioactive cytidine, was examined by quantitative DNA-RNA hybridization tests, autoradiography, and sedimentation analysis. About 60 percent of the radioactive RNA consists of 38 to 45S molecules located in the nucleoli and capable, under certain circumstances, of forming hybrids with DNA. Unlabeled 28S and 18S ribosomal RNA can compete with these components for DNA sites, indicating that the conversion of nucleolar RNA to ribosomal RNA occurs in such a way that base sequences are preserved. The polydisperse components associated with the chromatin region contain base sequences different from ribosomal and 4S RNA, and consequently have a uniquely high specific activity. These components are the only detectable hybridizing species in unfractionated preparations of RNA.*

In most cells rapidly labeled RNA appears at two distinct sites: the nucleoli and the extranucleolar or chromatin portions of the nucleus. The RNA labeled in the nucleolar region consists of large 38 and 45*S* molecules, the nucleotides of which ultimately become part of the 18 and 28*S* ribosomal RNA (*1–4*). Kinetic studies and base composition analyses (*3, 4*) suggest that a direct conversion, 45*S* → 38*S* → ribosomal RNA, occurs. What remains to be shown is that the large precursor molecules actually contain reasonably long base sequences identical to those of ribosomal RNA.

Chromatin-RNA is made up of a 4*S* component and of polydisperse components sedimenting between 8*S* and 45*S* (*1, 2*). The polydisperse components, metabolically unstable in the absence of concomitant ribosomal RNA synthesis, were suspected of being messenger RNA's. Although rapidly labeled RNA complementary to DNA (*3, 5*) or having a DNA-like base composition (*6*) has been described for mammalian cells, a definite identification with the chromatin fraction has not been made.

In this report we seek to characterize further the various rapidly labeled RNA fractions by studying their ability to form specific hybrid molecules with DNA and by observing whether there is a competitive effect of ribosomal RNA on the hybridization. Such techniques made possible the detection of DNA sequences complementary to the various kinds of bacterial RNA (*7, 8*). To distinguish between the rapidly labeled nucleolar and chromatin fractions we make use of the fact that cells exposed to low concentrations of actinomycin D synthesize normal amounts of RNA in the chromatin region, but have a greatly reduced capacity to make nucleolar RNA (*9*).