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Le Pecq J B & Paoletti C. A fluorescent complex between ethidium bromide and nucleic acids: physical-chemical characterization. *J. Mol. Biol.* 27:87-106, 1967.
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This paper describes the interaction of a trypanocidal drug, ethidium bromide, with nucleic acids. It is shown that this compound binds specifically to double stranded nucleic acids by intercalating its planar aromatic ring between two adjacent base pairs according to a model first proposed by L.S. Lerman.[1] The binding of ethidium to DNA and RNA is accompanied by a very large increase of its fluorescence quantum yield. It can therefore be used as a fluorescent probe of nucleic acid structure. [The *SCI*® indicates that this paper has been cited in over 690 publications since 1967.]

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"In the early 1960s, a lot of people were interested in studying the interaction of nucleic acids with various ligands such as simple cations, dyes, mutagens, and proteins. Known DNA-binding ligands, such as acridines, were often compounds endowed with pharmacological properties. Therefore, we thought it could be of interest to check whether drugs or biologically active substances having structures related to that of acridines could also be DNA ligands. We then noticed the papers of Dickinson *et al.*[2] and Newton[3] which reported that the trypanocidal action of phenanthridine drugs could result from interference with nucleic acid metabolism. We then asked Dickinson from Boots Pure Drug Company for samples of these compounds. We received a few milligrams of several derivatives. The most abundant sample was ethidium. I therefore began my study with this compound, which appeared later to be the most interesting one.

"I performed very simple experiments to start with, using differential spectroscopy to get evidence of DNA complexes. I immediately noticed that ethidium was able to complex with DNA because of the change of color of ethidium upon mixing with DNA. One day, it occurred to me that the ethidium-DNA mixture was shining a little bit. I therefore thought that ethidium could become fluorescent after DNA binding. At once, I prepared solutions of ethidium with and without DNA and went down three floors to another laboratory which had a fluorometer. Before doing any measurements, I put the cuvettes under the exciting beam and watched. I saw at once that the ethidium-DNA mixture emitted an intense, beautiful, orange-red fluorescence whereas the fluorescence of ethidium alone could hardly be seen with the naked eye. I immediately realized that this observation could be very useful and started quantitative fluorescence measurements the same day. It later appeared that, indeed, fluorescence was a very sensitive technique for characterizing the DNA binding of ethidium. Because of its fluorescent properties, ethidium became a widely used fluorescent probe of nucleic acids. It is commonly used to stain DNA bands in gel electrophoresis to detect them by fluorescence. Many citations to this work are probably related to the fluorescence properties of ethidium.

"At the time I wrote this paper, I moved to the California Institute of Technology for a postdoctoral stay with Davidson. I met Bauer and Vinograd whose labs were next to mine. I introduced ethidium to them. They were looking for intercalating reagents to study closed circular DNA and they were interested in ethidium properties. From their work,[4] ethidium became a widely used compound in this field, mainly to prepare circular DNA by ultracentrifugation in CsCl density gradient. A review has recently discussed the main uses of ethidium in the field of nucleic acid research."[5,6]

1. **Lerman L S.** Structural considerations in the interaction of DNA and acridines. *J. Mol. Biol.* 3:18-30, 1961. (Cited 935 times.) [*Citation Classic* in press.]
2. **Dickinson L, Chantrill B H, Inkley G N & Thompson M J.** The antiviral action of phenanthridinium compounds. *Brit. J. Pharmacol.* 8:139-42, 1953. (Cited 20 times since 1955.)
3. **Newton B A.** The mode of action of phenanthridines: the effect of ethidium bromide on cell division and nucleic acid synthesis. *J. Gen. Microbiol.* 17:718-30. 1957. (Cited 85 times since 1957.)
4. **Bauer W & Vinograd J.** The interaction of closed circular DNA with intercalative dyes. I. The superhelix density of SV40 DNA in the presence and absence of dye. *J. Mol. Biol.* 33:141-72, 1968. (Cited 435 times.)
5. **Morgan A R, Lee J S, Pulleyblank D E, Murray N J & Evans D H.** Ethidium fluorescence assays. Part I. Physicochemical studies. *Nucl. Acid. Res.* 7:547-69, 1979.
6. **Morgan A R, Evans D H, Lee J S & Pulleyblank D E.** Ethidium fluorescence assays. Part II. Enzymatic studies and DNA-protein interactions. *Nucl. Acid. Res.* 7:571-94, 1979.

