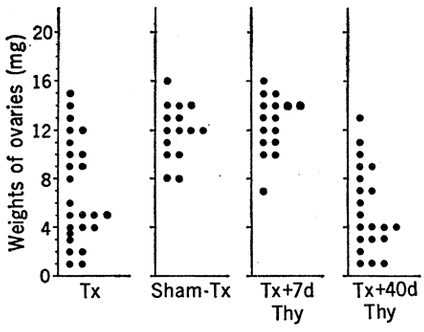

Fig. 3. Recovery of ovarian atrophy by thymus grafting in (C3H/HeMs × 129/J)F_1 mice; *Tx*, thymectomized at 3 days of age; *Sham-Tx*, sham-thymectomized at 3 days of age; *Tx+7dThy*, thymectomized at 3 days of age and grafted with newborn thymus at 7 days; *Tx+40dThy*, thymectomized at 3 days of age and grafted with newborn thymus at 40 days. All the mice were killed at 120 days of age. Each symbol indicates total weight of both ovaries in each animal.

change in the ovary could be recognized in the mice splenectomized at this age.

Examination on fertility of (C3H × 129)F_1 thymectomized mice showed that 36 out of 48 operated females (75 percent) were sterile, and the ovaries were constantly small and atrophic (at autopsy) at the age of 180 days. In these animals irregularity of estrous cycles—absence of proestrous and estrous stages—was seen on vaginal smears. In 20 males that were thymectomized at 3 days of age, no decreased capacity of fertility was found.

To account for the data that 25 to 40 percent of animals do not show the ovarian dysgenesia after thymectomy at 3 days of age, we searched for ectopic thymus outside the mediastinum; ectopy was frequently detected within the thyroid of BALB/c mice (*5*). Thirty-five female (BALB/c × 129/J)F_1 mice were thymectomized, as described above, and killed at the age of 360 days. Fifteen mice (43 percent) had normal ovaries (> 8 mg), and the remaining 20 had atropic ovaries (< 7 mg). Serial sections of the thyroid were made on 22 mice, randomly selected, and thymic tissue was found in the parathyroid area in ten mice whose ovaries were normal in size (> 8 mg) and histology. The remaining 12 mice with atropic ovaries (< 5 mg) had no ectopic thymus. This was also true for the (C3H × 129)F_1 mice thymectomized at 3 days of age: eight mice with normal ovaries had ectopic thymus and ten with atrophic ovaries had no ectopic thymus.

The next experiments were concerned with the effect of thymus replacement (Fig. 3). Mice of (C3H × 129)F_1 and (129/J × C57B1/6J)F_1 hybrids received thymectomy at 3 days of age which was followed by grafting at 7 days of age with one whole intact thymus from 1- or 7-day-old female or male donors into the No. 4 fat pad. All these mice had ovaries of normal size and morphology at subsequent autopsy (120 days of age). On the contrary, no recovery of the ovarian changes was attained in the thymectomized mice similarly grafted with thymus at the age of 40 days. These data indicate that the thymus grafts, only if implanted shortly after the thymus removal, can prevent ovarian dysgenesia.

Correlation between the gonad dysgenesia and immunologic function after thymectomy at 3 days of age was investigated. In both males and females of (C3H × 129)F_1 hybrid tested at the ages of 120 and 180 days, the thymectomy resulted in a moderate lowering of circulating lymphocytes, considerable decrease in number of plaque-forming cells in the spleen as determined by the technique of Jerne and Nordin (*6*), and a slight, borderline prolongation of the survival of allogenic skin grafts. No significant difference in these immunologic responses could be found between the thymectomized mice with normal ovaries and those with atrophic ovaries. Therefore, there is still no positive evidence that sex-linked developmental failure of the gonad is essentially related to depressed immunologic faculty after thymectomy at 3 days of age.

In view of our results, it seems plausible to propose that the thymus has the newly described function of controlling the reproductive faculty in the female mouse. However, we should consider the possibility that viral infection—mostly affecting the ovary after the thymectomy—may be a cause of the ovarian changes.

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3 July 1969

Berberine: Complex with DNA

Abstract. *A complex of calf-thymus DNA with berberine sediments in the analytical ultracentrifuge. The DNA produced systematic changes in the absorption spectrum of berberine which suggest that single alkaloid molecules bind to DNA. Flow dichroism of purines and pyrimidines and of berberine in the complex with DNA had the same signs and magnitudes. Berberine shifted the thermal strand separation profile of DNA to higher temperatures. Therefore, the alkaloid forms a complex with DNA, probably by intercalation.*

Among substances which form complexes with DNA are basic dyes (*1*), numerous antibiotics (*2*), and synthetic drugs (*3*). We have shown that a medically important alkaloid, quinine, forms a complex with DNA (*4*) and report here studies on a second example of interaction of an antimicrobial alkaloid, berberine, with DNA.

Berberine is widely distributed in higher plants (*5*). The alkaloid has antibacterial (*6*) and antiprotozoal (*7*) activity. By optical methods, berberine has been shown to interact with nucleic acids (*8*, *9*), especially DNA (*10*). Like acridines (*11*) or ethidium bromide (*12*), berberine converts yeast to respiratory-deficient cells which grow in "petite" colonies (*13*).

Table 1. Flow dichroism of the DNA-berberine complex. The method has been described previously (*15*, *16*). Dichroism is expressed as fractional change in absorbancy when the complex was flow oriented. The DNA concentration was 2 mg/ml; berberine, $2 \times 10^{-4} M$; and tris(hydroxymethyl)aminomethane-HCl buffer, $5 \times 10^{-3} M$ at *p*H 7.5. The light path was 0.25 mm; 0°, plane of polarized light parallel to axis of flow; 90°, plane of polarized light perpendicular to axis of flow.

Dichroism	259 nm	350 nm
At 0°	− 0.22	− 0.18
At 90°	+ .09	+ .08

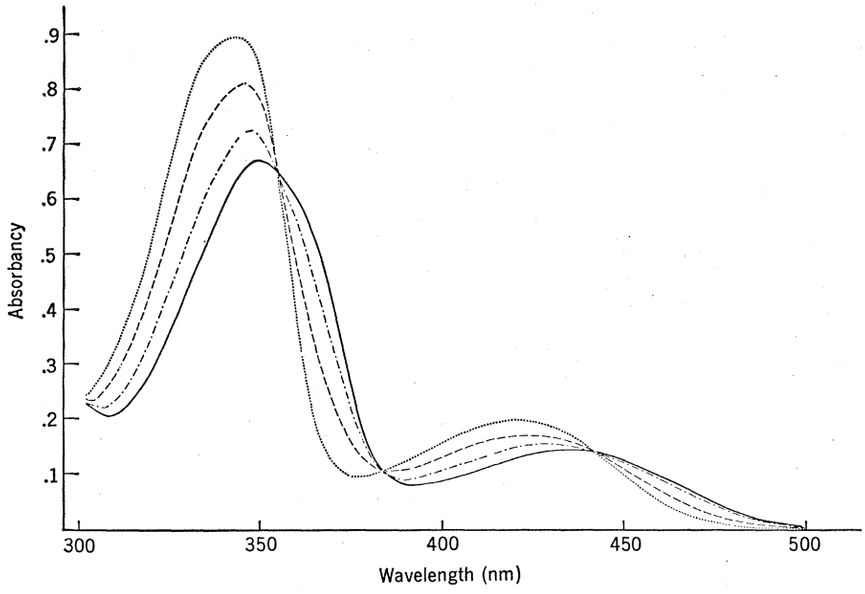


Fig. 1. Influence of DNA upon the absorption spectrum of berberine, $4 \times 10^{-5}M$ in $5 \times 10^{-3}M$ tris(hydroxymethyl)aminomethane-hydrochloride (tris-HCl) at *p*H 7.5. Berberine alone (• • • • •); berberine plus $1.14 \times 10^{-4}M$ DNA phosphorus (– – – – –); berberine plus $2.27 \times 10^{-4}M$ DNA phosphorus (– • – • –); berberine plus $4.54 \times 10^{-4}M$ DNA phosphorus (————). Calf thymus DNA was a commercial preparation (Calbiochem). Optical path, 1 cm.

The existence of a complex of DNA with a ligand can be simply demonstrated by showing that the complex sediments in the analytical ultracentrifuge. Berberine has one absorption maximum at 420 nm (Fig. 1). When a solution of calf thymus DNA (2 mg/ml) in berberine chloride ($10^{-4}M$) was centrifuged in a Spinco model E analytical ultracentrifuge at 59,780 rev/min, visual observation showed that the yellow berberine sedimented with the DNA accumulating below the hypersharp boundary.

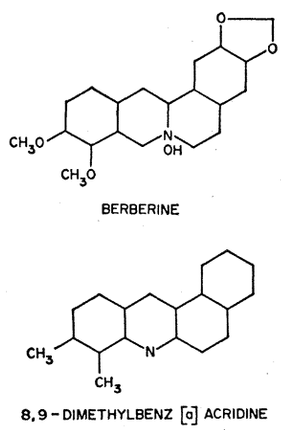
Figure 1 depicts the absorption spectrum of berberine alone at wavelengths longer than 300 nm and in the presence of graded concentrations of DNA. Increasing concentrations of DNA progressively decreased the absorption intensity of the two bands of the berberine spectrum. All absorption spectra passed through three discrete isosbestic points, an indication of the presence of only one spectrophotometric species of ligand bound to DNA in addition to free berberine. Furthermore, the progressive shift of absorption maxima in the direction of longer wavelengths with increasing concentrations of DNA suggests that berberine is bound to DNA as single molecules and, in this condition, exhibits a true "monomer" spectrum (*1*); we have, indeed, found that solutions of berberine alone show a comparable red shift in absorption bands upon progressive dilution. Evidently, unbound berberine has a marked tendency to self-aggregation.

The DNA-berberine complex was unstable in high concentrations of urea and in low concentrations of CsCl; $6M$ urea partly, and $3 \times 10^{-1}M$ CsCl almost completely, reversed the changes in the absorption spectrum of berberine induced by DNA. Apparently, berberine reacts with DNA through electrostatic attraction as well as through formation of hydrogen bonds.

The chemical structure of berberine is shown in Fig. 2. The molecule is planar with an estimated area of >50 Å^2, corresponding roughly to the area occupied by a base pair in double-helical DNA. A structurally reminiscent compound, 8,9-dimethylbenz[*a*]-acridine (Fig. 2), has been shown to enhance the viscosity of DNA (*14*) due to intercalation of the ring system between base pairs.

Intercalation presupposes that the planes of intercalated molecules lie parallel to those of purine-pyrimidine pairs in double-helical DNA. This was first shown experimentally by Lerman (*15*) for the acridine ring system of quinacrine and subsequently for chloroquine and various dyes (*16*). In analogous experiments, we oriented the DNA-berberine complex by flow and measured the flow dichroism at 259 nm for the purines and pyrimidines and at 350 nm for berberine. When dichroism was expressed as the fractional change in absorbance produced by flow orientation, the numbers in Table 1 were obtained. The signs and magnitudes of flow dichroism were the same for the constituent bases of DNA and for berberine. We conclude that in the complex the plane of the berberine molecule lies parallel to those of the base pairs of DNA.

Berberine stabilized DNA to thermal strand separation. Figure 3 shows the thermal strand separation profile of calf thymus DNA in the absence and in the presence of $5 \times 10^{-5}M$ berberine. The "melting" curve of the DNA-berberine complex was steeper than that of DNA alone, suggesting a more cooperative type of melting. A similar observa-



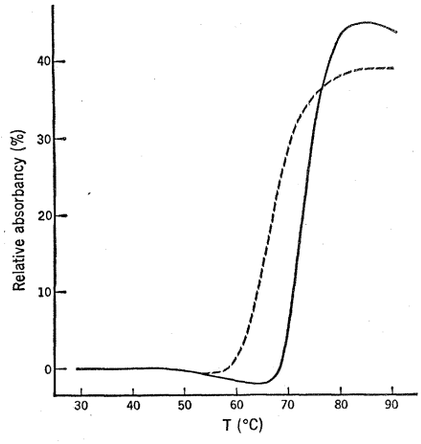


Fig. 2 (left). Chemical structures of berberine and of 8,9-dimethylbenz[*a*]acridine. Fig. 3 (right). Thermal dissociation of DNA in the presence of $5 \times 10^{-5}M$ berberine (————) and in its absence (– – – –); 20 μg/ml of DNA were dissolved in $5 \times 10^{-3}M$ tris-HCl at *p*H 7.5. Relative changes in absorbancies at 260 nm were calculated with respect to the absorbancy of DNA alone at room temperature.

tion has been reported for the influence of chloroquine upon the melting of DNA (*17*).

Intercalation into helical duplex structures is not the only type of binding of berberine to polynucleotides. Changes in the absorption spectrum of berberine are also induced by RNA (*8*), and enhancement of the fluorescence of the alkaloid is produced not only by double-helical DNA but also by denatured DNA and ribosomal RNA (*9*). These effects are not merely results of the binding of berberine to polyanions; interaction with chondroitin sulfate, a high-molecular (5×10^3 to 5×10^4 daltons) polyanionic polysaccharide, produces only weak enhancement of berberine's fluorescence (*9*). Monoribonucleotides do not enhance this fluorescence (*9*).

A biological effect of berberine is the conversion of yeast to respiratory-deficient cells which grow in "petite" colonies (*13*). This phenomenon has recently been studied in detail for ethidium bromide with the conclusion that this converting agent is intercalated into supercoiled mitochondrial DNA and produces configurational changes in this DNA which are responsible for the "mitochondrial mutation" (*12*). It is worthy of consideration that berberine acts in an analogous manner.

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31 July 1969

Host Finding by Odor in the Myrmecophilic Beetle Atemeles pubicollis Bris. (Staphylinidae)

Abstract. *The beetle* Atemeles publicollis *orients to the odor of its host ants by positive anemotaxis and osmoclinotaxis. The chemical response changes with age of the beetles. After hatching they are attracted by* Myrmica *odors, and, after hibernating, by* Formica *odors.*

Atemeles pubicollis, a staphylinid beetle, lives as a guest in colonies of ants. Soon after hatching in a nest of its first host *Formica polyctena* Foerst. the beetle migrates to and hibernates in a nest of its second host *Myrmica* sp. In the spring, *Atemeles* returns to the nest of *Formica*, where it reproduces. Although this life cycle was discovered almost 60 years ago (*1*), it remained unknown how the guests find their hosts' nests.

Laboratory experiments combined with field studies on the life cycle of *Atemeles* yielded the following results. Six to ten days after hatching, *Atemeles* leave the nest of *Formica*. Positive phototaxis and a high locomotor and flight activity lead them out of the ants' dark nest mounts and out of the wood biotope. Its second host *Myrmica* has its colonies in open grassland. However, in this biotope many other species of ants occur also. The myrmecophilic beetles cannot recognize the nests of *Myrmica* by optical cues because the nests are all situated either below the surface or under stones. The entrances of *Myrmica* nests look very similar to those of many other ant species.

Therefore, the involvement of olfactory orientation was investigated. Many ant species of the subfamily Myrmicinae mark their trails by pheromones (*2*). Some myrmecophiles living among army ants (*3*), and also the beetle *Amphotis marginata* Fabr. (*4*), a guest of *Lasius fuliginosus* Latr., can follow the trail pheromones of their hosts. *Atemeles*, in contrast, does not respond to trails of *Myrmica* when tested in the laboratory. Colonies of *Myrmica laevinodis* Nyl., *Lasius niger* L., *Camponotus ligniperda* Latr., and *Formica fusca* L. were placed around an arena, with the exits of these colonies covered by nylon netting which made it possible for the beetles to establish odor contact with the ants. In none of these experiments were beetles attracted to the exits of any of the colonies. If, however, weak air currents (0.5 m/sec) were blown through a nest of *Myrmica laevinodis* Nyl. and through *Formica fusca* L. and *Lasius niger* L. nests into the arena,

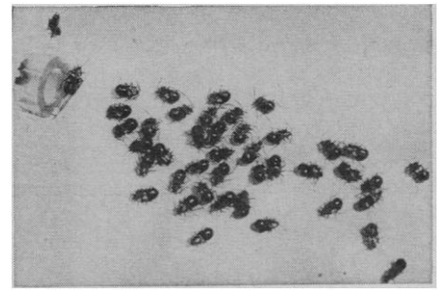

Fig. 1 (top right). *Atemeles pubicollis* follow an air current carrying the odor of their host ants' nest. They thus reach the nest entrance.

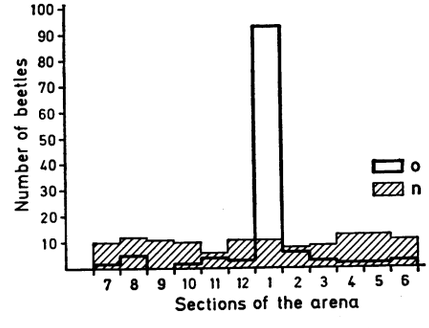

Fig. 2 (bottom right). Distribution of *Atemeles* in an arena when three different ant odors, carried by air currents, are offered simultaneously. The arena is divided into 12 sections. Ants' nest entrances are situated in sections 1 (*Myrmica laevinodis*), 4 (*Formica fusca*), and 10 (*Lasius niger*). Distribution of beetles, when no air is blown into the arena (*n*); distribution when odors are offered (*o*).