appreciable amount in incubated tumor homogenate, whether or not the homogenate is added to kynurenine (chromatograms No. 7 and No. 9).

The blockage of the ring opening and the blockage of the steps following the kynurenine pathway of tryptophan metabolism, reported here, indicate, in tumors, a marked decrease of tryptophan metabolism via kynurenine. A similar blockage of the ring opening was observed for histidine in hepatoma (*14*).

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Eastern Equine Encephalomyelitis Virus Isolated from Three Species of Diptera from Georgia

The virus of eastern equine encephalomyelitis has been isolated from Diptera four times since Kelser demonstrated that *Aedes aegypti* could transmit the virus in 1933. The significance of these isolations has been obscured by the difficulty in demonstrating experimental transmission with the species involved (*1*). Few would consider a species of insect a natural vector of a virus unless the virus could be isolated from it during an epizootic and unless the virus could also be transmitted experimentally by it.

The virus of eastern equine encephalomyelitis was isolated from pools of three dipterans: *Aedes mitchellae* (Dyar), *Anopheles crucians* Wiedemann, and an unknown species of *Culicoides* that were captured in southern Georgia in July 1956 (*2*). The isolation from *Aedes mitchellae* is probably most significant for species of this genus of mosquitoes have been effective experimental vectors of the virus (*3*). Chamberlain *et al.* (*4*) determined the threshold of infection, the transmission rate, and the infection rate of 20 species of mosquitoes. Excellent vector potentials were shown by three species, all belonging to the genus *Aedes*. *Anopheles crucians* was rated poor. *Culicoides* were not tested.

The mosquitoes and midges were collected in modified New Jersey type light traps on farms where horses had eastern equine encephalomyelitis (*5*). The live insects were anesthetized by chloroform and identified as to species in the case of mosquitoes and to genus in the case of *Culicoides*. Pools of one to 20 individuals were immediately ground in Ten Broeck tissue grinders with 1 ml of sterile, distilled water containing 5000 international units of penicillin with 5 mg of streptomycin. The resultant suspensions were centrifuged, and the supernatant fluids were inoculated into the allantoic chamber of 8-day embryonated chicken eggs, six eggs usually being used per inoculum, and each egg receiving 0.1 ml of fluid (*6*). Following inoculation, the eggs were incubated at a temperature of 35°C for 8 days. Allantoic and amniotic fluids were harvested from all embryos that died within this period and tested for bacterial contamination on nutrient agar and in thioglycollate broth. Harvests that appeared to be bacteriologically sterile were inoculated in further series of eggs. Isolates were sent to our laboratory in Wisconsin, where they were identified by titration with and without specific antiserum.

The pool of *Aedes mitchellae* from which virus was isolated was collected on 28 July from a farm near the town of Patterson in Pierce County. Eastern equine encephalomyelitis virus was isolated from the brains of two horses on this farm. The pool of *Anopheles cricians* was collected 30 July, on a farm in Appling County. The *Culicoides* from which the isolation was made were collected 28 July on a farm in Wayne County. Virus was recovered from a horse on this farm. The isolation history is given in Table 1.

The initial inoculum of the infected insect tissue killed half or more of the embryos after an unusually long incubation period of 69 to 144 hours. The period was reduced in the second or third passages to the characteristic time of 18 to 23 hours. The culture from *Anopheles* took four passages before all embryos were killed, and during this period the virus appeared to be sensitive to the effect of dilution and to freezing and thawing. Virus diluted in broth alone had a titer of $10^{3.5}$ as compared with its titer of $10^{5.8}$ when it was diluted in normal serum and broth. The adapted virus of the third or fourth passage of all three isolates possessed an embryo lethal titer of $10^{5.2}$ to $10^{6.2}$. Specific eastern equine encephalomyelitis antiserums prepared in chickens neutralized $10^{1.7}$ to $10^{2.8}$ LD_{50} of virus. Normal chicken serums and western equine encephalomyelitis antiserums did not neutralize the isolates.

All three dipterans from which the isolations were made are common in Georgia but are not widely distributed in other parts of the United States (*7*). *Aedes mitchellae* seems to be limited largely to the Atlantic and Gulf coastal plains, but, unlike its salt-marsh relative *Aedes sollicitans*, it breeds in fresh-water pools. Adults and larvae are seen throughout the year in southern Georgia. The range of *Anopheles crucians* is similar to that of *Aedes mitchellae*, but it extends further north and south, having been reported from Massachusetts and Central America. Its greatest abundance is reached in the cypress swamps of Georgia and Florida, where the larvae thrive in the acid waters of the swamps. Female *Anopheles crucians* are indistinguishable from *A. bradleyi* and *A. georgianus*. *Culicoides* are prevalent along the eastern seaboard in the tidewater counties where outbreaks of eastern equine encephalomyelitis have occurred most frequently.

Although nothing has been published about *Culicoides* and equine encephalomyelitis virus, the vector efficiency of the genus has been demonstrated for the blue tongue virus of sheep, and *Culicoides* are reported to transmit African horse sickness and fowl pox viruses (*8*). Robert Levi-Castillo of the Public

Table 1. Isolation and identification.

Source	Passage history									
	First		Second		Third		Titration	Neutralization		
	Mortality (No.)	Incubation (hr)	Mortality (No.)	Incubation (hr)	Mortality (No.)	Incubation (hr)		EEE	WEE	N
Aedes mitchellae	3/6	144–180	2/3	40–72	3/3	18–22	5.2	1.7	0	0
Culicoides spp.	4/6	136–180	2/3	23			6.2	2.7		
Anopheles crucians	3/3	69–144	4/20	24–96	1/10	24	5.8*	2.8	0	*

* Titer with normal serum; see text.

Health Service of Ecuador has described (*9*) the isolation of Venezuelan equine encephalomyelitis from *Culicoides*. The outbreak in Ecuador involved both men and horses.

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Effect of Gravity on Flowering of Soybeans

The antagonistic effects of synthetic auxin on the flowering of short-day plants have been demonstrated by numerous workers. High levels of natural auxin within plants have also been shown to delay flowering. Fisher and Loomis (*1*) and Fisher (*2*) suggested that high concentrations of naturally produced auxin at the lower nodes of soybean are antagonistic to floral induction. They found that, with plants on long photoperiods, flowering could be induced earlier by removing young, auxin-producing leaves after 3 to 5 trifoliate leaves had fully expanded. The complete loss of auxin-producing tissue through detopping, however, allowed active vegetative growth of the axillary buds. Such growth gave rise to high auxin levels at the lower nodes, thereby markedly delaying flowering.

Van Overbeek and Cruzada (*3*) showed that pineapple plants that were tipped on their sides flowered earlier than those that were grown upright. Pineapples do not behave like most short-day plants, since in them auxin has been shown to induce, rather than to inhibit, flowering (*4*). Apparently, then, the high auxin content in the apex of the horizontally grown plants induced earlier flowering, the auxin being concentrated in the apical regions by gravitational force. It was therefore thought that in soybeans, plants that begin to flower at the lower nodes, a similar method of growth might also cause an accumulation of auxin at the tip and subsequent lower auxin levels at the basal nodes, which would allow earlier floral induction.

Flambeau soybeans, on 18-hour photoperiods, were treated as follows: (i) in a control series, plants were allowed to grow normally; (ii) to make the plants grow downward, lead weights were placed around the stems near the tips of plants that had two mature trifoliate leaves; and (iii) lead weights were placed around the stems, near the tips, as in treatment ii, but vegetative suckers were removed as soon as they were 1 cm long. As the tips grew, the lead weights used in treatments ii and iii were moved toward the apex on the inverted stems. By the time seven or eight trifoliate leaves had fully expanded, 25 g of lead was required to keep the tips of the plants from turning upward. As the plants became older, the tips of the stems showed symptoms typical of the injury induced by an excess of externally applied auxin. Cellular enlargement and proliferation in the cortex were marked. The leaves continued to position themselves normally, resulting in a twisting of the petiole close to the stem. Enlargement of the petiole was pronounced in leaves that appeared after the sixth leaf was mature. Suckers usually grew from nodes 2 and 3.

Inverted plants flowered earlier and at lower nodes (Table 1) than the controls. Removing the suckers from the inverted plants stimulated the earliest flowering.

Table 1. Flowering of Flambeau soybeans on 18-hour photoperiods.

Treatment	Percentage flowering (days)					Lowest flowering node
	45	50	55	60	65	
Control	0	0	0	58	100	6.9
Inverted	0	44	55	78	100	4.7
Inverted, suckers removed	22	67	78	100	100	4.0

These data show that flowering in soybeans can be geotropically influenced, and they provide further support for a theory of auxin control in flowering. Apparently the accumulation of auxin in the tip region, through gravitational force, caused a reduced level of auxin at the basal nodes, and thereby induced earlier flowering (*5*).

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Triplet States of Biologically Active Molecules

In a recent communication concerning the probable importance of excited-state mechanisms in biological systems, and in particular the role of triplet-state energy transfer in oriented or partly oriented aqueous media, A. Szent-Györgyi (*1*) suggests that the mode of action of many drugs may involve interference with energy-transfer processes. As evidence for this possibility, Szent-Györgyi cites the fact that 2-4-dinitrophenol is physiologically active at similar concentrations to those at which it will quench the phosphorescence of riboflavin; he also cites other very suggestive but not conclusive *in vitro* experiments on the fluorescence of aqueous dyestuffs.

In this connection, I wish to report some observations made in 1953 but not yet published, on phosphorescence from narcotized tissue. We had been investigating (*2*) sensitive methods of detecting carcinogens by low-temperature fluorescence spectroscopy and the *in vivo* conditions of formation of carcinogen-protein complexes. We decided to look briefly at the low-temperature emission spectra obtainable from spontaneous tumor tissue. Aqueous tissue homogenates and ether extracts crystallized in an excess of naphthalene (which provided ordered host material) were prepared, and the emission spectra were observed at 90°K under irradiation from a mercury arc. The samples used were human tumor tissue from 15 to 20 patients. All