that the duration of recording of bursts of potentials was fixed, so that with changes in stimulus frequency the number of stimuli in each burst also was changed.

Three types of results are shown in Fig. 1. The points in C23 connected by the heavy line represent values of the amplitude of potentials evoked at each of a range of frequencies, in a cat which gave a response at each presentation of the electrical stimulus, there being no more than one false-positive response in each ten trials and no negative responses. The points connected by the broken line represent values obtained from the same cat and with the same stimulus parameters after free feeding, until the animal no longer responded to the electrical stimulus, but did so to other events in its environment, for example, the approach of another cat. The demonstrated change in the form of the curve did not take place immediately after satiation but rather after an additional half-hour of repeated negative trials.

The points in C21 connected by the heavy line represent values of amplitude from another cat in the same circumstances as in C23. The points connected by the broken line represent values obtained after extinction of the response by omitting the reward. Again, this change did not follow immediately upon cessation of response but after repeated negative trials.

The points in C27 connected with the solid line represent values from one of the control cats after elicitation of the orienting response with a strong electrical stimulus, the stimulus intensity thereafter having been reduced to 1.3 times threshold for the electrical response. The points connected by the broken line represent values obtained after 6 days of repeated electrical stimulation with habituation; that is, the cat was in the same environment as C23 and C21 but with no significance being attached to the stimulus.

The dotted curve in each of the three examples was derived from the equation describing forced harmonic motion in a system driven by a sinusoidal force:

$$m\frac{d^2x}{dt^2} + r\frac{dx}{dt} + kx = F\cos\omega t$$

As applied to an electronic oscillator, the constant m would represent inductance, r resistance, and k the reciprocal of capacitance. Although each of these elements has been described in squid axon (*5*), no physical meaning was assigned to them in the present study. The equation was used in one of its integrated forms:

$$E = \frac{F}{\omega\{r^2 + [(k/\omega) - \omega m]^2\}^{\frac{1}{2}}} + C$$

where E represents amplitude, F the driving force (dependent only in part on stimulus intensity), ω the stimulus frequency times 2π, and C an arbitrary constant of unknown significance. Use of the equation in this form implied two approximations: (i) that despite the shortness of the bursts (115 msec) the amplitude represented predominantly the steady state rather than the transient response; and (ii) that the driving force was sinusoidal, whereas in actuality it was impulsive. By these assumptions calculations were avoided, which were prohibitively complex for this preliminary stage of analysis.

These results show that the empirically derived relationship between the amplitude of evoked potential and the frequency of electrical stimulation corresponds to the analytical function describing forced harmonic motion, in states wherein the stimulus influences overt behavior at intensities well below convulsion levels. It may be inferred that when the cat is attentive to a stimulus, the cortex upon which it falls becomes (in a formal sense) resonant to that stimulus. In states of habituation, satiation, extinction, and also sleep, which alike imply the probability of inattention, the correspondence is not apparent. The change in form of the curves is compatible less with increased damping than with some disorganization of the cortex manifested by the appearance of two peaks of amplitude in the place of one.

The agreement between the observed and calculated data implies that the periodic activity of the cortex is harmonic, but it does not specify the mechanism, that is, whether the property of oscillation is inherent in the physicochemical structure of single neurons or in chains of neurons forming reverberating circuits. However, it does specify the need for further studies of the resistive and reactive properties of neurons and neuroglia, as well as comparison of the frequency-specificity of the excitability change with amplitude changes previously found to accompany changes in attention (*6*).

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13 February 1961

Isolation of Viruses from Children with Infectious Hepatitis

Abstract. Cytopathologic agents were isolated from 14 of 22 Indian children involved in an outbreak of infectious hepatitis in Arizona. Isolation was made in a serially transplantable cell line originating from human embryonic lung. Efforts to identify these agents as known viruses, by utilizing standard techniques, have been unsuccessful.

A serially transplantable cell line highly susceptible to viral infections was isolated from human embryonic lung during studies on the etiology of viral gastroenteritis. The superiority of these cells over established lines in primary isolation of enteroviruses (*1*) prompted attempts to isolate viruses from the feces of patients with clinical symptoms of infectious hepatitis.

In October 1959 an epidemic of infectious hepatitis was observed on an Indian reservation in eastern Arizona. There were 22 hospitalized cases of this disease in children varying in age between 20 mo and 3 yr. The course of the disease was severe, and protracted hospitalization was required in a majority of the cases. Scleral icterus was noted in all cases, although dermal icterus was not pronounced because of dark pigmentation. All urines were strongly positive for bile pigments, serum icterus indexes were elevated, and stools usually were clay colored. The clinical diagnosis of all 22 children was infectious hepatitis.

Tissue cultures were prepared as outlined previously (*1*) and changed to a maintenance medium of 2 percent calf serum and M199 prior to inoculation of the fecal specimens. Twenty percent fecal suspensions were prepared for inoculation into tissue culture according to methods described by Paul (*2*). One-tenth of a milliliter of 20 percent fecal suspension and 0.9 ml of maintenance medium were

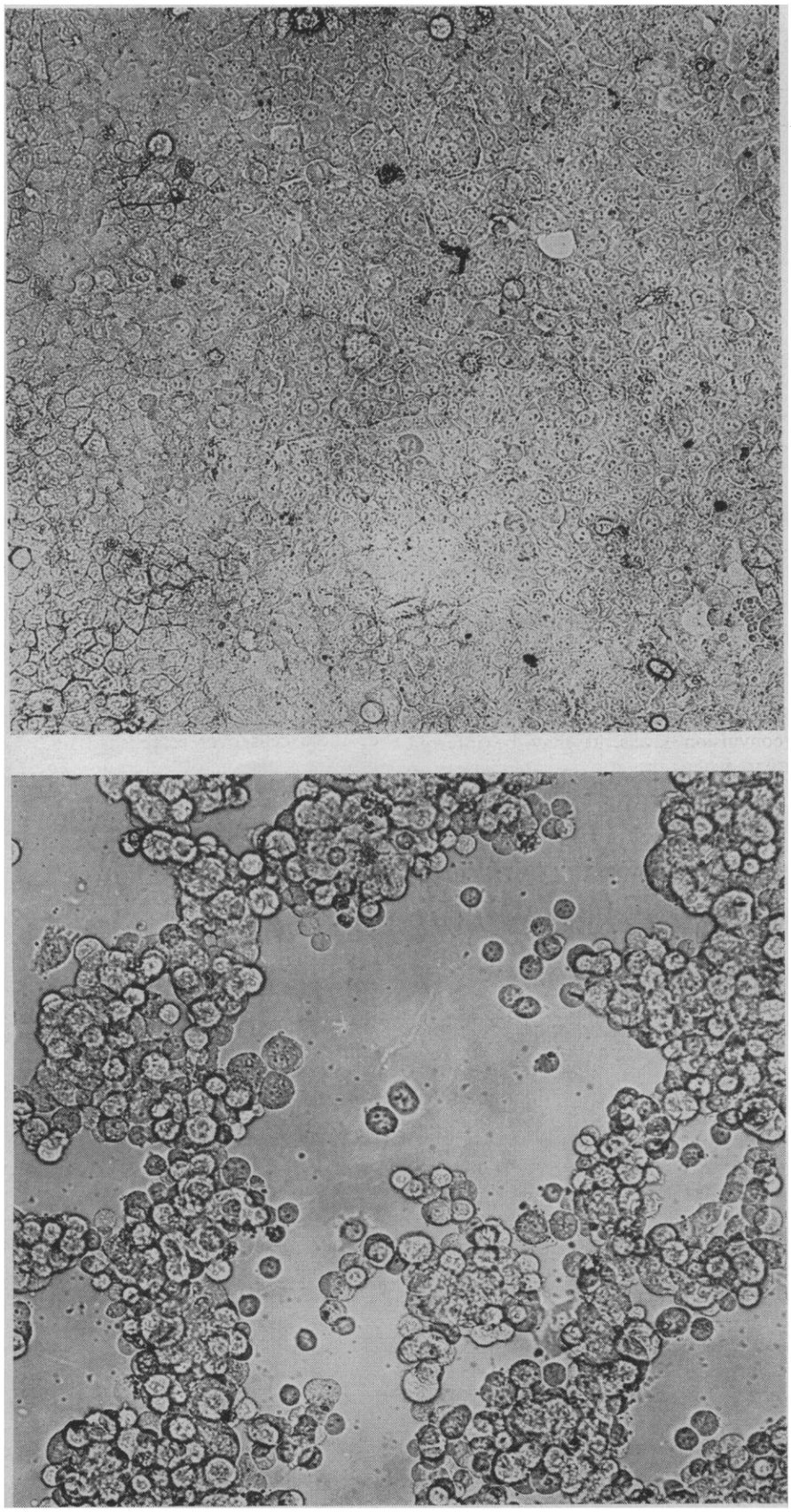

Fig. 1. (Top) Living culture of embryonic human lung cells. (Bottom) Same culture, 72 hr after infection, showing typical cytopathology of the Arizona viruses isolated.

added to each culture tube. Incubation was carried out at 37°C in a standard roller-tube apparatus.

First passages were characterized by marked phagocytosis of colloidal fecal particles producing a generalized cytoplasmic granularity. Viral cytopathogenicity, however, was indicated on the 5th day after infection by rounding, ballooning, and eventual clumping of infected cells. The clumping observed was strikingly characteristic of an agglutination-type reaction. Subsequent passages eliminated granularity and showed a consistent viral cytopathic effect (see Fig. 1).

Serially transplantable cytopathologic agents were isolated from 14 of the 22 children examined. Only one isolation was made from 23 persons without apparent symptoms of infectious hepatitis. These latter persons, ranging in age from 1½ mo to 66 yr, were familial contacts of the acute cases. The single isolate was from a 2-yr-old sibling of an acute case. Specimens taken from one of two children with clinical diagnosis of diarrhea in the same hospital yielded a virus indistinguishable from those isolated in the overt hepatitis cases.

With a single exception, attempts to isolate these viruses in primary renal epithelial culture of *Macacus rhesus* were unsuccessful after three serial passages.

Antisera against known enteroviruses (*3*)—Echoviruses 1-28, Coxsackie B viruses 1-6, Coxsackie virus A-9, and three poliovirus types—failed to neutralize against 10, 100, 1000 $TCID_{50}$ of each isolate.

Suckling mice remained well when inoculated intracerebrally, intramuscularly, or intraperitoneally; similarly, the viruses introduced into the allantoic or amniotic cavities of 7-day embryonated eggs were without effect. No demonstrable infection was observed when the isolates were placed on the scarified cornea of adult albino rabbits. Complement fixation, with adenovirus antiserum, was undetectable at serum dilutions of ¼ and ⅛. Hemadsorption or hemagglutination could not be detected when using guinea pig, human cells from blood types A, B, and O, or adult or 1-day-old chicken erythrocytes. Hemagglutination of *Macacus rhesus* erythrocytes occurred sporadically and appeared to depend on the animal from which the cells were

obtained. The growth pattern of the viruses is unlike that of cytomegaloviruses (*4*).

The agents survive a temperature of 60°C for 40 min. Infectivity titers for embryonic human lung cultures vary from $10^{-3.5}$ to $10^{-5.5}$. Cross protection tests with convalescent sera indicated a serologic relationship between 13 of the 14 isolates. The only exception was the virus which grew in the monkey renal cultures. Acute sera from four of the hospitalized patients failed to neutralize homologous viral isolates, whereas sera from 1-yr convalescents neutralized 100 to 1000 $TCID_{50}$ of virus at serum dilutions of 1/25 and 1/50. Sera obtained from two adults who had recovered from infectious hepatitis 1 yr previously neutralized all the viruses except the monkey kidney isolate. The neutralization index with the latter sera was as great as that of convalescent sera from hospitalized cases (*5*).

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5. Efforts to further characterize these isolates and studies on their epidemiology are being advanced.

13 March 1961

Gibberellin as Sex Regulator in Ricinus communis

Abstract. Spraying monoecious inbreds with gibberellin A_3 can markedly increase their female tendency. The effect of this substance upon sex appears to simulate the action of long days.

The castor bean, *Ricinus communis* L., is a monoecious, potentially everflowering perennial. The main stem, which blooms first, and all subsequent shoots, are terminated by racemes, and each raceme differentiates both staminate and pistillate flowers. The percentage of pistillate flowers in the racemes of individual plants is taken as a measure of their female tendency. This character is subject to extreme genetic and nongenetic variations.

Table 1. Effect of spraying seedlings with gibberellin upon the female tendency of a monoecious inbred of the castor bean. Figures in parentheses refer to the total number of flowers. The spraying regimes are given in the text.

Treatment	Individual plants employed	Pistillate flowers (%) in sequential racemes			
		First	Second	Third	Fourth
		Sprayed			
A	108-1	86.5(37)	100.0(41)	92.7(55)	34.7(101)
A	108-2	87.9(83)	100.0(85)	73.9(85)	73.9(130)
B	108-3	66.0(97)	97.6(42)	44.7(132)	40.8(49)
B	108-4	74.6(63)	88.7(53)	59.4(69)	36.3(113)
C	108-5	57.9(107)	73.8(84)	38.6(132)	21.1(109)
C	108-6	61.2(67)	93.5(46)	38.0(108)	26.2(130)
		Unsprayed			
	108-7	27.4(72)	15.8(101)	12.9(109)	29.4(167)
	108-8	32.8(67)	24.2(62)	14.7(75)	14.9(134)
	108-9	31.7(83)	19.7(71)	18.4(76)	25.6(78)
	108-10	25.7(105)	24.3(66)	15.8(54)	25.8(159)
	108-11	32.1(84)	18.2(88)	13.2(91)	27.1(151)
	108-12	27.3(77)	25.9(81)	17.4(115)	24.5(188)

In connection with a study of the relationship between female tendency and the incidence of female mutants in monoecious inbreds (*1*, *2*), a search was made for substances which might regulate sex expression in these inbreds.

Previous investigations demonstrated that in monoecious inbreds of cucumbers, *Cucumis sativus* L., certain treatments with 1-naphthalene acetic acid (NAA) increase (*3*) and certain treatments with gibberellic acid or gibberellin (GA) decrease (*4*) female tendency. It is also known that staminate flowers can be induced by GA in some genetic females of cucumbers (*5*, *6*). These findings prompted us to test NAA and GA as possible sex regulators in the castor bean.

Various spraying regimes with NAA at concentrations ranging from 10 to 100 parts per million (ppm) did not affect female tendency. On the other hand, spraying with GA markedly increased female tendency (Table 1). In the experiment with GA, I used an early-flowering monoecious inbred No. 108 which was self-reproduced for 11 generations. This inbred was originated from a selection made in the variety Gamadon (*1*). The seed was germinated at 30°C on 8 August 1960, and 12 uniform plants were grown in 12-in. pots, in soil, under greenhouse conditions, without supplementary light, at day temperatures of 24° to 27°C and night temperatures of 18° to 21°C (thermostatically controlled). The sprayed and unsprayed plants were randomized.

The sprays used were aqueous solutions of Merck's Gibrel, a potassium salt of gibberellin A_3. Each treated plant was sprayed three times—at the two-leaf, three-leaf, and four-leaf stages of growth, successively. In treatment A, the concentrations of the gibberellin solutions employed were 1500 ppm for the first spraying, 1500 ppm for the second, and 500 ppm for the third. In treatment B, the corresponding concentrations were 1500, 1000, and 500 ppm; and in treatment C, they were 1500, 500, and 125 ppm (Table 1). The spraying began on 26 August and the first racemes appeared on 8 September. In both sprayed and unsprayed plants the number of leaves to the first raceme on the main stem ranged from six to eight and averaged seven.

By selective pruning of side shoots it was possible to obtain four sequential racemes on each plant, and these racemes were developmentally comparable in all plants.

As is shown in Table 1, the first three sequential racemes on each of the six sprayed plants were greatly affected by the GA treatments. One should also note the trend in the differences between treatments A, B, and C.

In the unsprayed plants (Table 1) there is a drop in female tendency from the first to the third raceme in each individual, and this drop is followed by a recovery in the fourth raceme. The racemes of the sprayed plants do not exhibit the same developmental change, presumably because of the cumulative effect of the three GA treatments at seedling stages. Cyclic changes in sex tendency during development are not uncommon in some untreated monoecious species of plants (*6*).

It is evident that GA is an effective substance for increasing female tendency in the castor bean. Other GA experiments with inbred No. 108, as well as with several distinctly different mono-