Table 3. Inhibition of RSV-transformation by ara-C. Various concentrations of ara-C were added for 16-hour intervals before or after exposure of cells to dilutions of RSV stock. Antiserum to RSV was added in all media used after infection, and deoxycytidine ($10^{-3}M$) was added to all mediums after removal of ara-C. Cultures were incubated for 1 week at 40°C under nutrient agar, and characteristic focuses of Rous sarcoma cells were counted. The numbers indicate resulting focuses. When no ara-C was added, there were 56,900.

Exposures to ara-C	Molarity of ara-C		
	$10^{-3.5}$	10^{-4}	$10^{-4.5}$
Before RSV	12,300	23,800	37,400
Immediately after RSV	360	550	1,120
16 hr after RSV	7,100	11,900	26,000

DNA synthesis in cultures of chick-embryo cells but has no effect on RNA synthesis over a 24-hour period (*5*). The inhibitory effect of ara-C can be prevented by deoxycytidine but not by cytidine (*5*), and cells exposed to ara-C resume DNA synthesis if ara-C is removed and deoxycytidine added (Table 1). Reversal of the effect of ara-C decreases if the interval between initial exposure and removal is extended beyond 8 to 10 hours and the capacity of cells to resume growth begins to decrease by 12 hours after initial exposure to ara-C.

Experiments dealing with transformation stemmed from the following observation on virus synthesis. Chick-embryo cells were exposed to RSV for 45 minutes, then washed with buffered saline. Cytosine arabinoside ($10^{-3.5}M$) was added, and 8 or 12 hours later the medium containing ara-C was replaced with medium containing deoxycytidine. The yield of virus 24 to 36 hours after infection was considerably reduced compared to that in untreated controls (Table 2). The reduction could not be attributed merely to an 8- or 12-hour delay in virus synthesis since virus production in untreated cultures after 24 hours was much greater than in treated cultures after 32 or 36 hours. This inhibition of DNA synthesis during infection with RSV may have resulted in an abortive infection, although the effect of a slight delay in resumption of DNA synthesis is difficult to assess. The effect of interruption of DNA synthesis on transformation of cells by RSV was examined.

Groups of chick-embryo cell cultures were exposed to various dilutions of RSV for 45 minutes and washed with buffered saline. After infection, antiserum to RSV was incorporated in all mediums to prevent elution and reinfection by superficially attached virus and to reduce complications of virus-yielding cells later in the course of incubation. Cultures were exposed to ara-C for 16-hour periods: 16 hours before infection, 0 to 16 hours after infection, or 16 to 32 hours after infection. These three intervals were necessary to assess the temporal effect of inhibition of DNA synthesis on subsequent morphological transformation of cells. After the removal of ara-C, cultures were washed with buffered saline, and growth medium containing deoxycytidine was added. At the 32nd hour, nutrient agar medium containing deoxycytidine was added, the cultures were incubated at 40°C for 1 week, and typical Rous sarcoma focuses were counted. The recorded focuses were calculated relative to an undiluted sample of infecting virus.

The presence of ara-C from 0 to 16 hours immediately after infection reduced the number of focuses to about 3 percent of cultures treated with ara-C before infection and to 5 percent of cultures treated 16 to 32 hours after infection (Table 3). The slight inhibition noted in cultures treated before infection compared to untreated controls can be attributed to the failure to reverse the effect of ara-C immediately upon addition of deoxycytidine (Table 1). The inhibition noted in cultures treated 16 to 32 hours after infection may be due in part to the asynchrony of the DNA-dependent phase of transformation among different cells. In all cases where ara-C is used, some inhibition of focus formation may be caused by the failure of a portion of the treated cells to resume growth. However, maximum inhibition of transformation occurred only in the group receiving ara-C immediately after infection. Therefore, the inhibition of DNA synthesis by ara-C has a specific effect on the transformation process, and the observation cannot be attributed to a generalized toxic effect on chick-embryo cells, or to an increased susceptibility of RSV-infected cells to the cytotoxic action of ara-C.

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References and Notes

1. H. V. Gelboin and M. Klein, *Science* **145**, 1321 (1964).
2. G. J. Todaro, *Proc. Amer. Assoc. Cancer Res.* **6**, 65 (1965).
3. J. P. Bader, *Virology* **22**, 462 (1964).
4. H. M. Temin, *ibid.* **23**, 486 (1964).
5. J. P. Bader, *ibid.* **26**, 253 (1965).
6. L. V. Crawford and E. M. Crawford, *ibid.* **13**, 227 (1961).
7. H. Hanafusa, T. Hanafusa, H. Rubin, *Proc. Nat. Acad. Sci. U.S.* **49**, 572 (1963).
8. J. P. Bader, *Virology* **16**, 436 (1962).

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Unique Sterol in the Ecology and Nutrition of Drosophila pachea

Abstract. Drosophila pachea, *which breeds only in the stems of senita cactus* (Lophocereus schottii) *throughout the Sonoran Desert, requires the cactus as a dietary supplement when reared on laboratory media.* Δ^7*-Stigmasten-3β-ol, isolated from the cactus or synthesized, can replace the cactus in the diet of flies reared nonaseptically or axenically.* Δ^7*-Cholesten-3β-ol and* $\Delta^{5,7}$*-cholestadien-3β-ol could be substituted for the cactus sterol;* $\Delta^{5,7}$*-stigmastadien-3β-ol produced infertile females. Cholesterol, 4α-methyl-*Δ^7*-cholesten-3β-ol, β-sitosterol, stigmasterol, ergosterol, and* Δ^7*-ergosten-3β-ol did not support larval growth.*

Drosophila pachea Patterson and Wheeler was described from two males and two females collected near Hermosillo, Sonora, Mexico, in August 1941 (*1*). The species would not breed on standard laboratory media for *Drosophila*, and therefore no culture was established at that time. The species was rediscovered in 1962 during a survey of the breeding sites of cactiphilic *Drosophila* in the Sonoran Desert and attracted our attention on two counts: first, *D. pachea* was bred only from stems of senita cactus, *Lophocereus schottii* (Engelmann) Britton and Rose, a columnar cactus abundant in the states of Sonora and Baja California, Mexico (Table 1); and second, no other species of *Drosophila* utilize the stems of this cactus for breeding purposes, even though two other species of local *Drosophila* have been reported to breed in the fruits of senita (*2*).

Our early tests showed that *D. pachea* could breed successfully on a standard medium composed of bananas, malt, corn syrup, and yeast only when the medium is supplemented with a cube of fresh or autoclaved senita cactus. Without the

Table 1. Emergence records for *Drosophila pachea* from fermenting stems of *Lophocereus schottii* in Mexico.

Locality*	Date	Substrate (g)	Flies (No.)
Desemboque	2- 4-1962	1140	63
Commondú	2-24-1962	268	6
Desemboque	5-25-1962	576	108
Tiburón Island	5-25-1962	901	3
Kino	5-26-1962	566	101
Sonoita	11-23-1962	1136	668
Lobos	1-21-1963	427	105
Esperanza	1-27-1964	956	255
Empalme	1-28-1964	235	103
Bisnaga	12- 2-1964		37
Guaymas	12-28-1964	164	10

* All localities are in the state of Sonora except Commondú, which is in Baja California.

cactus, larvae sometimes start development, but they usually die in the second instar. Moreover, by the same type of test, senita cactus was toxic in varying degrees to adults and larvae of other species of local *Drosophila* (Table 2). In this table, each test and control represents three to four transfers through a 30-day period of the original adults in large standard shell vials. All progeny of the four species, however, no matter how few survived, were fertile.

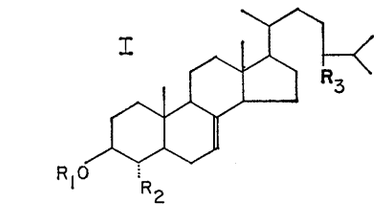
The preliminary tests then support the deduction from field observations that the senita cactus contains a factor (or factors) necessary for the development of *D. pachea* and a factor (or factors) which tends to exclude other species. The remainder of this report is concerned with the identification of the factor necessary for growth.

Senita cactus contains several alkaloids (*3*) as well as two uncommon sterols (*4*), 4α-methyl-Δ^7-cholesten-3β-ol [lophenol (I*a*, Fig. 1), 0.23 percent of dry cactus] and Δ^7-stigmasten-3β-ol [schottenol (I*b*, Fig. 1), 0.13 percent of dry cactus]. However, not all of the physical constants of schottenol and its derivatives were identical with those of Δ^7-stigmasten-3β-ol and its derivatives (*5*), and therefore only a tentative assignment of the structure of schottenol as Δ^7-stigmasten-3β-ol was made (*4*) (Fig. 1).

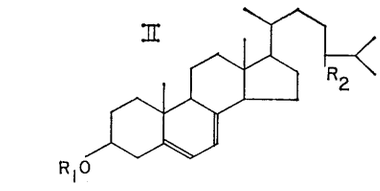
In order to determine if the sterols are necessary for growth of *D. pachea*, they were isolated from the cactus, separated on a silica-gel column with a mixture of petroleum ether, ether, and acetic acid (120 : 40 : 1), and purified by the procedure used by Djerassi (*4*). Lophenol, m.p. 145° to 146°C [compared to 149° to 151°C (*4*)], and schottenol, m.p. 151° to 152°C [compared to 149° to 150°C (*4*)], separated very well on thin-layer plates of silica gel (solvent: petroleum ether-ethyl acetate, 60:40), with an R_F corresponding to that of authentic samples. Our samples were dissolved in ether and added in varying amounts to vials containing 15 ml of the standard banana medium. The ether was removed with stirring on a steam bath and the vials were cooled. A total of 20 females and 15 to 20 males of 1-week-old specimens of *D. pachea* obtained from Sonoita, Sonora, were placed in each vial; all concentrations of each sterol and the combined sterols were tested in duplicate. The controls consisted of four vials of banana medium supplemented with a cube of senita cactus and four vials of straight banana medium. The adults were not removed from the vials until the media were well worked with larvae.

Lophenol (I*a*) will not support growth even though many larvae started development; the number of progeny from schottenol (I*b*) appears to be regulated by the concentration of the sterol in the medium (Table 3). The fertility of the progeny was tested by transferring all the offspring F_1 (except those grown on 0.01 percent schottenol plus lophenol vials) to a banana medium supplemented with senita cactus. The F_1 from all concentrations produced many larvae except those grown on 0.01 percent schottenol; they produced fewer larvae, many of which died. Thus, *D. pachea* requires schottenol and cannot use lophenol.

In order to establish the structure of schottenol as Δ^7-stigmasten-3β-ol (I*b*), the compound was synthesized by another method. β-Sitosteryl acetate was brominated with *N*-bromosuccinimide and dehydrobrominated with collidine in xylene (*6*) to $\Delta^{5,7}$-stigmastadien-3β-ol acetate (II*a*), which was reduced with hydrogen over Raney nickel (*7*) in ethanol-ether; the reduction product, Δ^7-stigmasten-3β-ol acetate (I*c*), m.p. 158° to 160°C, was hydrolyzed to Δ^7-stigmasten-3β-ol (m.p. and mixed m.p.



Structure	R_1	R_2	R_3
I*a*	H	CH_3	H
I*b*	H	H	C_2H_5
I*c*	CH_3CO	H	C_2H_5
I*d*	H	H	H
I*e*	H	H	CH_3



Structure	R_1	R_2
II*a*	CH_3CO	C_2H_5
II*b*	H	H
II*c*	H	C_2H_5

Figs. 1 (top) and 2 (bottom). Sterol structure related to schottenol.

151° to 153°C), whose infrared spectrum was superimposable on that obtained from the schottenol isolated from the cactus (Fig. 2).

When the synthesized material was added to the standard banana medium, it elicited the same response as did the schottenol isolated from senita cactus.

To exclude the possibility that microorganisms may act as an intermediate in the utilization of schottenol, a substrain of *D. pachea* was prepared under aseptic conditions. Eggs laid by fertile adults on wet, killed yeast were rinsed from the yeast with a dilute detergent solution (Renex) into a 100-mesh copper-wire basket (*8*). The detergent was washed from the eggs with 80 percent ethanol, and the basket was immersed in this solvent for 20 minutes and then in sterile distilled water for 10 minutes. The eggs were then transferred to vials containing autoclaved enriched medi-

Table 2. Death rate and reproduction in four species of *Drosophila* on standard banana medium supplemented with a cube of sterile senita cactus.

Species	Adults				Total No. of progeny	
	Original No.		No. after 15 days			
	Test	Control	Test	Control	Test	Control
D. melanopalpa	23	24	10	20	7	398
D. hydei	30	30	25	25	62	892
D. pseudoobscura	30	30	4	29	182	309
D. simulans	58	57	48	45	711	831

Table 3. Average number of progeny per culture vial from 35 to 40 parents. The percentage of sterol was based on dry ingredients in medium.

Growth supporters	Percentage of sterol in medium			
	0.5	0.1	0.05	0.01
Schottenol	162	106	69	63
Schottenol + lophenol	251	181	114	5
Lophenol	4 pupae	0	0	0
Medium + senita cactus			148	
Medium alone			0	

* Each sterol was added at the indicated concentration; for example, 0.5 percent schottenol and 0.5 percent lophenol.

um (*9*) supplemented with senita cactus and lacking the mold inhibitor; they were allowed to develop normally through the larval stage (9 to 14 days) to imagos (18 to 21 days). The population has been maintained axenically for 7 months by careful transfer of adults to autoclaved vials containing the enriched medium and senita cactus. The latter has now been supplanted with synthetic schottenol. Contamination soon becomes evident and such cultures are discarded. The axenic culture was tested periodically for contamination by microorganisms. In the presence of senita cactus or schottenol, *D. pachea* mature and reproduce on the aseptic medium, an indication that microorganisms do not participate in the utilization of the sterol by the fly.

To determine whether other sterols can be substituted for schottenol in the diet of *D. pachea*, the standard banana medium was supplemented with a number of sterols (Table 4). The concentration of added sterols was 0.5 percent of the dry diet or 7 mg per 15 ml of medium. Under these conditions, nonaseptic, fertile flies laid eggs and the larvae hatched. After 16 days the adults that were still alive were removed; only four out of the ten sterols supported growth (Table 4).

The F_1 offspring from flies grown on these four sterols were each subsequently divided into three groups. One group was placed on the banana medium supplemented with senita cactus, another was placed on the medium supplemented with the sterol from which the F_1 offspring were reared, and the third group was placed on the banana medium without supplementation. In all of the senita tests, many F_2 adults developed. Many F_2 adults also developed in media containing schottenol, Δ^7-cholesten-3β-ol, and $\Delta^{5,7}$-cholestadien-3β-ol, but none were produced in media containing $\Delta^{5,7}$-stigmastadien-3β-ol. In fact, no eggs were laid by the F_1 reared from this sterol, except in the senita test. Dissection of some of the flies showed that the males contained motile sperm and that the females were inseminated but contained immature eggs. When the sibs of these flies were subsequently placed on medium supplemented with senita cactus, the females became fertile, laid eggs, and produced a large number of progeny.

In Table 4, notice that the F_2 from standard banana medium (last column in the table), whose parents had been raised on Δ^7-cholesten-3β-ol and $\Delta^{5,7}$-cholestadien-3β-ol, produced no progeny when placed on the standard medium. Reproductive ability, therefore, which is dependent on the presence of certain sterols, cannot be transferred more than one generation in the absence of these sterols.

Table 4. Nonaseptic *D. pachea* on standard banana medium supplemented with various sterols; the number of progeny are given from two culture tubes for each sterol.

P_1 Adults placed on medium plus:	Culture after 16 days	Total F_1 offspring after 33 days	Total F_2 offspring from F_1 placed on standard medium plus:		
			Senita cactus	Sterol on which F_1 were reared	No sterol
Cholesterol*	Dead larvae	0			
β-Sitosterol*	Dead larvae	0			
Stigmasterol*	Dead larvae	2			
Lophenol (*Ia*)	Dead larvae	1			
Schottenol (*Ib*)	Pupae	260	Many	Many	2
Δ^7-Cholesten-3β-ol (*Id*)*	Pupae	270	Many	Many	24‖
Δ^7-Ergosten-3β-ol (*Ie*)†	Dead larvae	2			
Ergosterol*	Dead larvae	0			
$\Delta^{5,7}$-Cholestadien-3β-ol (*IIb*)‡	Pupae	250	Many	Many	29‖
$\Delta^{5,7}$-Stigmastadien-3β-ol (*IIc*)§	Pupae	175	Many	No eggs	No eggs
No sterol	Dead larvae	4			

* Purchased from commercial sources. † Raney nickel hydrogenation of ergosterol; m.p. 149°–150°C [149°–150°C (*15*)]. ‡ Prepared from cholesterol; m.p. 148°–149.5°C [149°–151.5°C (*6*)]. § Intermediate in schottenol synthesis; m.p. 150.5°–151°C [144°–145°C (*16*)]. ‖ When tested on standard banana medium, these adults produced no progeny.

The importance of sterols to insects has been reviewed (*10*); every insect that has been investigated to date requires a sterol in its diet, and, in all cases reported, cholesterol satisfies this requirement. Therefore *D. pachea* is the first species described that cannot use this sterol. Van't Hoog (*11*), working with *D. melanogaster*, showed that β-sitosterol, ergosterol, and stigmasterol can be substituted for cholesterol in the diet. However, *D. pachea* can use none of these.

The function of sterols in the diets of those insects tested is at least twofold: they are the probable precursors of the molting hormone, ecdysone (*12*), and in some way control the fertility of the female (*10*). With *D. pachea*, larval growth and maturation were produced by Δ^7-cholesten-3β-ol (I*d*), $\Delta^{5,7}$-cholestadien-3β-ol (II*b*), Δ^7-stigmasten-3β-ol (schottenol, I*b*), and $\Delta^{5,7}$-stigmastadien-3β-ol (II*c*), but only the first three of these four sterols yielded fertile females. This suggests that the growth and molting hormone or hormones can arise from all four sterols, but that the material controlling egg maturation in the female cannot be derived from the last-named sterol. It is also of interest that *D. pachea* could use neither ergosterol nor Δ^7-ergosten-3β-ol (I*e*). Possibly de-ethylation, but not demethylation, can occur at C-24 in the sterol molecule in *D. pachea* to yield cholestane derivatives. De-alkylation at C-24 occurs in both the ergostane and stigmastane series in *Blattella germanica* (*10*).

Drosophila pachea, therefore, lacks enzyme systems that are present, at least, in some other insects. It is unable to convert Δ^5-3β-sterols or C-24 methyl substituted sterols to metabolically active compounds. It has a dietary requirement for a sterol with a hydrogen atom or an ethyl group at C-24 and a double bond in the Δ^7 position. An additional double bond in the Δ^5 position can be tolerated in the cholestane series, but in the stigmastane series it leads to nonfertile females.

Wagner (*13*) indirectly demonstrated the importance of yeasts in supplying a sterol in the fruits of the cactus (*Opuntia Lindheimeri*) for growth of *D. aldrichi* and *D. mulleri*. By contrast, *D. pachea* live in fermenting pockets in the arms of the senita cactus, where schottenol is in high concentration and where larval growth does not appear to depend upon the microbial synthesis of sterols.

Drosophila aldrichi and *D. mulleri*

are members of the large desert-adapted *repleta* species group to which also *D. pachea* originally had been assigned (*1*). However, a more recent evaluation by L. Throckmorton on the basis of internal anatomy shows that *D. pachea* is more closely related to *D. nannoptera*, a desert-inhabiting species from southern Mexico. The *nannoptera* species group, which is monotypic, is considered phylogenetically older than the *repleta* group (*14*). *D. pachea*, therefore, apparently has had ample time to evolve into and become dependent upon a niche which supplies it with a unique sterol. This niche in the Sonoran Desert is secured from competitors by the presence of another substance which is toxic in varying degrees to other species of local *Drosophila* and to which *D. pachea* has evolved a tolerance. Our results indicate that the alkaloid fraction of the cactus contains this material.

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References and Notes

1. J. T. Patterson, *Texas Univ. Publ. No. 4313* (1943).
2. ——— and W. S. Stone, *Evolution in the Genus Drosophila* (Macmillan, New York, 1952), p. 610.
3. C. Djerassi, S. K. Figdor, J. M. Bobbitt, F. X. Markley, *J. Amer. Chem. Soc.* **79**, 2203 (1957); C. Djerassi, T. Nakano, J. M. Bobbitt, *Tetrahedron* **2**, 58 (1958); C. Djerassi, H. W. Brewer, C. Clark, L. J. Durham, *J. Amer. Chem. Soc.* **84**, 3210 (1962).
4. C. Djerassi, G. W. Krakower, A. J. Lemin, H. H. Liu, J. S. Mills, R. Villetti, *J. Amer. Chem. Soc.* **80**, 6284 (1958).
5. D. H. R. Barton and J. D. Cox, *J. Chem. Soc.* **1948**, 1354 (1948).
6. S. Bernstein, L. J. Binovi, L. Dorfman, K. J. Sax, Y. Subbarow, *J. Org. Chem.* **14**, 433 (1949).
7. G. D. Lauback and K. J. Brunings, *J. Amer. Chem. Soc.* **74**, 705 (1952); W. V. Ruyle, E. M. Chamberlin, J. M. Chemerda, G. E. Sita, L. M. Aliminosa, R. L. Erickson, *ibid.*, p. 5929.
8. P. Graf, M.S. thesis, University of Arizona, 1965.
9. M. R. Wheeler and F. Clayton, *Drosophila Information Service* **40**, 98 (1965).
10. R. B. Clayton, *J. Lipid Res.* **5**, 3 (1964).
11. E. G. van't Hoog, *Z. Vitaminforsch.* **5**, 118 (1936).
12. P. Karlson and H. Hoffmeister, *Z. Physiol. Chem.* **331**, 298 (1963).
13. R. P. Wagner, *Texas Univ. Publ. No. 4445* (1944), p. 104.
14. L. H. Throckmorton, *Texas Univ. Publ. No. 6205* (1962), p. 207.
15. P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch, G. W. Wood, *J. Chem. Soc.* **1951**, 2402 (1951).
16. W. Wunderlich, *Z. Physiol. Chem.* **241**, 122 (1936).
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Background and Evoked Activity in the Auditory Pathway: Effects of Noise-Shock Pairing

Abstract. *Unanesthetized cats with electrodes permanently implanted along the auditory pathway were presented first with sustained "white" noise stimuli and then with the same noise paired with subcutaneous electric shocks. Pairing noise with shock decreased both background and noise-evoked activity in the inferior colliculus, and, in some cats, also in the cochlear nucleus, trapezoid body, and at the round window. No changes occurred in medial geniculate or auditory cortex recordings. The effects in the inferior colliculus do not depend on changes in the degree of arousal of the animal, on changes in the medullary auditory areas, or on the actions of the middle-ear muscle.*

It is well known that perceptions are influenced by expectations, purposes, and past experience (*1*). The neurological mechanisms underlying these effects have traditionally been assigned to "associative" or "integrative" areas of the brain; the sensory systems were thought to simply relay information about the physical characteristics of the stimulus from receptor to primary cortex (*2*). However, neuroanatomists have demonstrated descending tracts in parallel with the classical ascending sensory pathways, and electrical stimulation of these descending systems modifies evoked potentials in the sensory nuclei (*3*). Therefore, it seems possible that past experience may affect perception by acting on sensory signals very early in the input pathway (*4*).

In order to investigate the plasticity of the subcortical auditory areas, cats were presented first with sustained noise stimuli and then with the same noise paired with subcutaneous electric shocks. Background and noise-evoked activity were recorded with permanently implanted electrodes. Sustained noise was used instead of the conventional click or tone pips, because Starr and Livingston (*5*) showed that the sustained response to sustained noise is limited to the classical auditory pathway, while click-evoked responses are widespread in the unanesthetized brain. This report describes the changes in electrical activity observed under these conditions.

Multiple electrodes were implanted in nine adult male cats, at the round window (RW), cochlear nucleus (CN), lateral trapezoid body (Trz), superior olive (SO), inferior colliculus (IC), medial geniculate (MG), and primary auditory cortex (AI) (Table 1). All electrode placements were verified histologically (*6*). The electrodes were made of 36- or 32-gauge insulated stainless steel wire with only the cut ends bare. The electrodes for RW were monopolar spirals (*7*); all other electrodes were bipolar, with tips 0.5 to 2.0 mm apart. Electrodes for delivery of conditioning shocks were implanted subcutaneously on the cat's back. The middle-ear muscles of all animals were

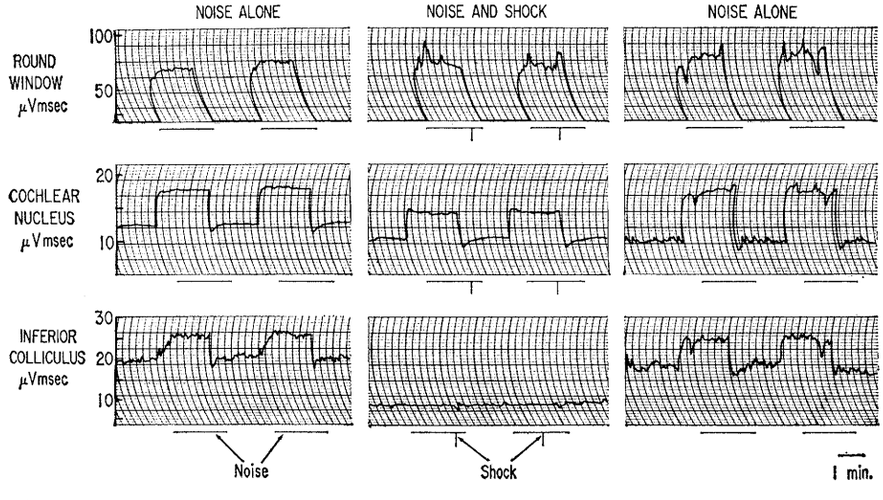

Fig. 1. Simultaneous "integrator" recordings from RW, CN, and IC. Samples from the 15th day on which noise was presented alone, the 2nd day on which both noise and shock were presented, and the 27th day of the second "noise-alone" period. Noise presentations (70 db) indicated by horizontal bars. Subcutaneous shocks indicated by vertical markers. Noise-shock pairing decreases background and evoked activity at IC although RW response is unchanged. The decrease in CN background persists after CN evoked activity has returned to the control level.