To test the RNA for possible biological activity, suspensions of fresh lymph node cells from "neutral" rabbits were divided into two equal aliquots. One aliquot was incubated in vitro at 37°C for 15 minutes with "reactive" RNA extracted from recipient rabbit popliteal lymph nodes draining a skin homograft. The RNA solution (concentration 80 to 400 μg/ml) was made 0.7*M* with respect to sucrose and the *p*H was adjusted to 7.6 prior to incubation with the cells. The other aliquot of cells from the same "neutral" rabbit was incubated under similar conditions in a similar hypertonic medium containing no RNA. After incubation, each aliquot was centrifuged, resuspended in tissue culture medium, and injected intradermally into opposite sides of the back of the rabbit which had donated the skin homograft. The results of these experiments are summarized in Table 2. It is apparent that in 17 of the 20 rabbits tested the aliquot of cells incubated with "reactive" RNA produced a significant reaction (2+ or greater). The 2+ reactions elicited by the untreated "neutral" cells in rabbits No. 105 and 146 remain unexplained and are the only instances in more than 100 intradermal injections wherein 4 to 10 million untreated "neutral" lymph node cells produced a significant skin reaction. The same experiment was repeated in seven rabbits using RNA from unstimulated "neutral" lymph nodes. No significant skin reactions were produced by cells incubated with such "neutral" RNA. We have similarly demonstrated that "reactive" RNA alone, when injected intradermally into the rabbit which had donated the homograft, failed to produce a reaction. It thus appears that a combination of "neutral" cells plus "reactive" RNA is necessary to produce a positive skin reaction under the conditions tested. We have further shown that prior treatment of the "reactive" RNA with ribonuclease will destroy its potential reactivity. Finally, we have shown that the reactions produced by "neutral" cells incubated with "reactive" RNA are completely specific for the donor of the skin homograft and will not occur when these cells are injected into a "neutral" rabbit (*6*).

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Biosynthesis of Coumarin and Herniarin in Lavender

Abstract. Coumarin and herniarin are formed in lavender from labeled *o*- and *p*-coumaric acids, respectively. At least part of the herniarin exists in lavender as a glycoside, presumably a 4-methoxycoumarinic acid glycoside, which yields free herniarin on hydrolysis. Biosynthesis of herniarin by successive *para*- and *ortho*-hydroxylation of cinnamic acid is indicated.

Several theories have been advanced to account for the biosynthesis of the 7-hydroxycoumarins (*1*), but experimental evidence about the mode of formation of these widely distributed coumarins has been meager. One of them, scopoletin, can be formed from labeled phenylalanine (*2*). I have recently suggested that some form of cinnamic acid, which can be formed enzymically from phenylalanine (*3*), first undergoes either *ortho*- or *para*-hydroxylation, with the eventual synthesis of coumarin or a 7-hydroxycoumarin, respectively (*4*). Although existing evidence lends support to this idea (*5*), no quantitative tracer studies have been done to permit a more rigorous delineation of the intermediates lying between phenylalanine and the 7-hydroxycoumarins.

Solutions of radioactive compounds were administered through the roots of *Lavandula officinalis* Chaix plants (fresh weights 30 to 55 g) which had been grown hydroponically under artificial illumination (*6*). The plants were grown for 6 to 7 days after activation, under the same conditions. Upon harvesting, they were immediately homogenized in hot ethanol, and after removal of the ethanol in a vacuum, free coumarin and herniarin (7-methoxycoumarin) were recovered by ether extraction of the aqueous residue. In these plants the amounts of free coumarins were usually very small, but when the aqueous solutions were then hydrolyzed with emulsin additional coumarin (36 to 77 mg) and herniarin (5 to 13 mg), as well as *o*-coumaric acid, could be recovered by ether extraction. The two coumarins were separated and purified by gas-liquid phase chromatography at 210°C on a column of succinic acid–ethylene glycol polyester (1 part) on Chromosorb W (20 parts).

The release of herniarin by emulsin is evidence for the existence in lavender of a glycoside of 4-methoxycoumarinic acid, a previously unreported compound. This glycoside has also been detected and partially purified by paper chromatography. Two analogous glycosides are known: coumarinyl glucoside,

Table 1. Conversion of C^{14}-labeled compounds to coumarins by lavender.

Compound administered	Specific activity (μc/mmole)	Compound isolated*	Specific activity (μc/mmole)	Dilution of C^{14}
		Experiment 1		
L-Phenylalanine-G-C^{14}	33.3	Coumarin	0.166	201
		Herniarin	0.31	108
		Experiment 2		
Glucose-G-C^{14}	100	Coumarin	0.015	6,700
		Herniarin	0.047	2,100
p-Coumaric acid-2-C^{14}	100	Coumarin	0.0022	45,000
		Herniarin	0.405	247
o-Coumaric acid-2-C^{14}	100	Coumarin	0.309	324
		Herniarin	0.0012	83,000
o-Coumaryl glucoside-2-C^{14}	100	Coumarin	0.755	132
		Herniarin	0.0004	250,000
		Experiment 3		
p-Coumaric acid-2-C^{14}	68	Coumarin	0.0015	45,000
		Herniarin	0.114	596
o-Coumaric acid-2-C^{14}	95	Coumarin	0.350	272
		Herniarin	0.0023	41,000

* Free coumarins in experiment 1, and coumarins liberated by hydrolysis in experiments 2 and 3.

and a glycoside in *Coronilla glauca* seeds which can be hydrolyzed to yield psoralene (*7*). The former yields coumarin not only on hydrolysis with emulsin, as above, but also with an endogenous β-glucosidase in *Melilotus* (*8*).

Table 1 shows the results of the tracer experiments. The results of experiment 1, with L-phenylalanine, support earlier findings (*2, 6, 9*) which indicate that this amino acid is a general precursor of coumarins. The appreciably higher specific activity of herniarin as opposed to coumarin suggests that the former did not arise through introduction of a methoxyl into the latter. The data from experiments 2 and 3 clearly show that *o*-coumaric acid and its glucoside were used with high specificity for the synthesis of coumarinyl glucoside, and that *p*-coumaric acid was used with similar specificity for synthesis of the glucoside which yields herniarin. Glucose-G-C^{14}, as expected, was used with lower efficiency for both syntheses.

The stage at which O-methylation takes place during the biosynthesis of 4-methoxycoumarinic acid glycoside is unknown. But the existence of this glycoside, and the fact that it is formed from *p*-coumaric but not *o*-coumaric acid, indicate the following partial biosynthetic sequence: (i) *para*-hydroxylation of a phenylpropanoid precursor (probably cinnamic acid), (ii) *ortho*-hydroxylation, and (iii) formation of a glycoside at the *ortho*-hydroxyl group. The results strongly suggest that lactone ring formation occurs, as in the case of coumarin (*4, 8*), simply by spontaneous dehydration after glycoside hydrolysis.

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Effects of Early Perceptual Restriction on Simple Visual Discrimination

Abstract. Dogs were raised from infancy to maturity in lighted cages that restricted their visual experience but did not deprive them of all patterned stimulation. After they were released from their cages, they had greater difficulty than normally reared littermates in performing a simple black-white discrimination and in subsequent reversal training.

Severe restriction of the early perceptual experience of dogs has profound effects on their behavior at maturity (*1*). One of the most striking results is their frequent failure to perceive noxious stimuli.

Scottish terriers raised in restriction cages often failed to show any responses indicative of pain (apart from reflex movements) when their noses were touched with flaming matches or when they were jabbed with a dissecting needle. Moreover, the Scotties banged their heads repeatedly on low-lying water pipes without showing any signs of pain. However, intense stimulation, such as strong electric shock, produced violent emotional disruption in all the dogs.

This abnormal pain perception suggests that perception in other sensory modalities may also be affected by early restriction. A study of the effects of early perceptual restriction on discrimination of simple visual stimuli was therefore carried out (*2*). The subjects were five pure-bred Beagle littermates. Two dogs ("Dulla" and "Sin") were raised in a restricted environment. Each was placed, at 3 weeks of age, in a specially constructed cage that permitted feeding and care but prevented the dog from making contact with the outside environment. Each cage was well-lit, so that the dogs were able to see visual patterns provided by the cage construction (lines, angles, circles, and rectangles) and their own bodies. But the variety of patterned stimuli, compared with that in a normal environment, was drastically reduced. Starting at 9 months of age, the two restricted dogs were released from their cages for 15-minute periods each day for general observation. The three dogs comprising the control group were raised normally on a farm until they were 9 months old. They were then brought to the laboratory, where they lived two in a cage and were frequently permitted to run in a large outdoor enclosure.

Visual discrimination training began 5 weeks after the restricted dogs were first released from their cages. The dogs, which were kept on a 24-hour food-deprivation schedule, were trained to run down an alleyway 4 feet long and to obtain food by pushing open one of two doors at the end of the alley. The door carrying the positive stimulus could be opened by a slight push; the door holding the negative stimulus was locked. Food was always present behind both doors. The dogs were usually returned to the start box immediately after they made an error; correction was permitted only on the first training problem and during black-white reversal training after a significant difference had been established. The dogs were subjected to ten trials a day until they achieved criterion on a given problem (18 correct responses out of 20 trials, given on two consecutive days).

The dogs were first trained to perform a visual brightness discrimination. They learned to run to the side lighted by a 60-watt bulb and to avoid the unlit side. There was no difference between the two groups. One control dog learned the discrimination after 10 days and the other two after 12 days; one of the restricted dogs learned after 7 days and the other after 12 days.

Striking differences were noted, however, when the dogs had to discriminate between a white card (positive stimulus) and a black card (negative stimulus) which were located on the doors (Fig. 1). The response patterns of the two restricted dogs were almost identical. Each showed rapid initial learning of the problem, presumably a transfer from the earlier brightness discrimination, which was followed by a rise in errors before they finally achieved criterion performance. The increase in errors was accompanied by vicarious trial and error behavior at the choice point, in which the dogs appeared suddenly to become aware of the cues provided by the cards on the doors. The control dogs, on the other hand, showed a smooth decrease in errors after the second day. The difference in error scores between the two groups is significant at better than the .05 level ($t = 3.08$).

The differences between the two groups were even more marked in reversal training, which was carried out 6 weeks later. The procedure was reversed so that the black card now signaled food and the white card was on the locked door (Fig. 2). The control dogs showed a gradual decrease in er-