ment of other EEG patterns or specific frequency bands as conditioning stimuli, and by using the spontaneous variations in electrical activity of diverse cerebral loci as signals. Using this technique, we not only detect relatively slight changes in consciousness associated with certain electrical patterns but may also be able to influence the frequency of occurrence of certain normal and abnormal patterns. Furthermore, these methods may permit conscious realization of shifts in cerebral electrical activity which herald not only epileptic seizures but perhaps other more subtle and commonplace alterations in cerebral activity.

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26 June 1962

Ribonucleic Acid in "Transformation" of Lymphoid Cells

Abstract. When incubated with "reactive" ribonucleic acid extracted from lymph nodes of rabbits immunized by skin homografts, "neutral" lymph node cells from nongrafted rabbits were apparently altered to a state of transplantation immunity, as manifested by a positive skin reaction upon injection of these cells into the donor of the skin homografts.

Alterations in cell metabolism after the incorporation of foreign nucleic acids have been convincingly demonstrated. However, most previous work has been concerned with bacterial transformation (*1*) and with investigation of infectious nucleic acids extracted from plant and animal viruses (*2–4*). Recent results obtained in our laboratory suggest that adult mammalian lymph node cells may be altered with respect to transplantation immunity by incubation with homologous lymphoid RNA obtained from immune animals. While the evidence presented below is insufficient to support the conclusion that the observed alterations in cellular reactivity have resulted from a process analogous to bacterial transformation, nevertheless the possibility that a form of transformation has taken place cannot be ruled out.

White New Zealand rabbits were used as experimental animals. As an indication of transplantation immunity, a skin test was utilized similar to the "transfer reaction" successfully employed by Brent, Brown, and Medawar (*5*) in guinea pigs. In brief, a suspension of 4 to 10 million fresh immune lymphoid cells, obtained 8 days after grafting from a recipient rabbit's popliteal lymph nodes draining a skin homograft, were injected intradermally into the back of the rabbit which had donated the homograft. The appearance after 48 to 72 hours of a tuberculin-like skin reaction at the injection site was considered a positive test. On each occasion the donor rabbit received an intradermal injection of an equal number of lymph node cells from a nongrafted "neutral" rabbit into the opposite side of the back as well. Thus, the reaction produced by the "neutral" cells and that produced by the immune cells, stimulated by the skin homograft, could be compared in each case. An arbitrary scale of 0 to 5+ was used in grading the reactions. The results obtained with this test in 13 donor rabbits are summarized in Table 1. It is clear that the immune cells in every case but one produced a significant (2+ or greater) skin reaction in the donor animals, and that "neutral" cells failed to produce any reaction greater than 1+ (smallest reaction definitely perceptible). As a check on the specificity of the skin test, an equal number of immune cells (4 to 10 million) were injected intradermally into a "neutral" rabbit in each experiment. In six of 13 experiments the immune cells produced a significant (2+ or greater) skin reaction in the neutral rabbit as well as in the donor of the skin homograft. We have interpreted this result as indicating the presence of common tissue antigens in both the "neutral" and donor rabbits in some instances.

Utilizing the same skin test as evidence for transplantation immunity, we have subsequently explored the possibility of transforming "neutral" lymphoid cells to a state of transplantation immunity. The RNA used in these experiments was extracted with phenol from a homogenate of fresh lymph node tissue suspended in citrate buffered saline. The extraction procedure was carried out in the cold following the modification by Alexander *et al.* (*4*) of the method of Gierer and Schramm (*2*). The product has an ultraviolet absorption spectrum characteristic of RNA. The hyperchromatic effect on incubation with NaOH is 31 percent. The diphenylamine test for DNA is negative. In the ultracentrifuge the material forms three peaks sedimenting at approximately S-21, S-8, and S-4, respectively.

Table 1. Maximal skin reactions produced by immune or "neutral" lymph node cells 48 to 72 hours after intradermal injection into the rabbits indicated. Intensity of reactions graded on scale of 0 to 5+ (see text). The symbol ± denotes the doubtful presence of a perceptible reaction.

Rabbit No.	Donor rabbit		Neutral rabbit (right side: immune cells)
	Right side: immune cells	Left side: "neutral" cells	
8	4+	0	0
17	4+	1+	1+
16	1+	0	0
35	2+	0	0
40	4+	1+	2+
129	2+	±	1+
42	3+	0	0
48	4+	0	2+
149	5+	0	3+
164	4+	1+	3+
170	5+	0	2+
154	3+	1+	1+
151	3+	0	2+

Table 2. Maximal skin reactions produced 48 to 72 hours after intradermal injection into donor rabbit by "neutral" lymph node cells incubated with "reactive" RNA or with media alone. Intensity of reactions graded on same scale as Table 1 (see text).

Rabbit No.	Right side: cells + RNA	Left side: cells + media
63	2+	1+
65	4+	0
64	4+	0
79	2+	0
100	1+	1+
277	2+	0
116	2+	0
124	2+	0
119	2+	1+
126	3+	0
133	4+	0
105	2+	2+
137	1+	0
139	4+	1+
135	2+	1+
148	3+	±
145	2+	0
146	3+	2+
150	3+	±
95	1+	0

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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6 July 1962

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.