females reject male grafts may be regarded as a ready-made source of "isogenic resistant" (*7*) animals, and this may have some practical application for immunological studies (*8*).

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References and Notes

1. E. J. Eichwald and C. R. Silmser, *Transplantation Bull.* 2, 148 (1955); E. J. Eichwald, C. R. Silmser, N. Wheeler, *Ann. N.Y. Acad. Sci.* 64, 737 (1957).
2. R. T. Prehn and J. M. Main, *Natl. Cancer Inst.* 17, 35 (1956); B. F. Short and W. R. Sobey, *Transplantation Bull.* 4, 110 (1957).
3. B. B. Hirsch, *Transplantation Bull.* 4, 58 (1957); M. Feldman, *ibid.* 5, 15 (1958).
4. P. B. Medawar, *J. Anat.* 78, 176 (1944).
5. E. J. Eichwald, C. R. Silmser, I. Weissman, *J. Natl. Cancer Inst.* 20, 563 (1958); S. E. Bernstein, A. A. Silvers, W. K. Silvers, *ibid.* 20, 577 (1958).
6. R. E. Billingham, L. Brent, P. B. Medawar, *Phil. Trans. Roy. Soc. London* 239B, 357 (1956); R. E. Billingham and L. Brent, *Transplantation Bull.* 4, 67 (1957).
7. G. D. Snell, *J. Genet.* 49, 87 (1948).
8. This investigation was supported by research grant C-3577 from the National Institutes of Health, U.S. Public Health Service.

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Effects of Intracerebral Injection of Anticholinesterase Drugs on Behavior in Rats

Krech, Rosenzweig, *et al.* have reported that the level of cholinesterase in localized brain areas correlates with the maze learning behavior of rats (*1*). Animals that utilized visual cues (visual hypothesis) to run an insoluble maze had a lower average cholinesterase activity in visual and somesthetic areas than those that followed spatial cues (spatial hypothesis). These workers proposed (i) that the cortical cholinesterase level is directly proportional to the rate of cortical acetylcholine metabolism; (ii) that a high level of acetylcholine metabolism implies a readier synaptic transmission of impulses, which is responsible for the more adaptive, probabilistic, spatial hypothesis; and (iii) that a low rate of acetylcholine metabolism results in a more stimulus-bound, visual hypothesis. An indirect test of these propositions was the finding that rats displayed increased visual hypotheses in maze running after intraperitoneal injection of small doses of pentobarbital sodium. This drug inhibits acetylcholine synthesis (*2*).

In the study reported here (*3*), attempts were made to test whether the cortical level of acetylcholine influences the maze running behavior of rats. Two anticholinesterase drugs, physostigmine (eserine) and diisopropyl fluorophosphate (DFP), were injected either directly into the visual or somesthetic cortex, or directly into one of the lateral ventricles. Such injections were expected to inhibit cerebral cholinesterase, leading to an increase in cortical acetylcholine. A subsequent shift from visual to spatial hypotheses in rats so treated would support the propositions set forth above.

Thirty-four naive male hooded rats, 3 to 4 months old, were used (*4*): 13 of the Tryon Maze-Bright strain S1 (spatial hypothesis), 13 of the Tryon Maze-Dull strain S3 (visual hypothesis), and 8 of a crossed strain between these two (S13). The experimental period for each rat ranged from 25 to 115 days. We used a four-unit Y-maze (*5*), and followed the training method and schedule of reinforcement used by Krech *et al.*

All animals were operated upon under ether anesthesia. Four 1/8-in. holes were made in the skull, bilaterally above the visual and somatic areas, for intracortical injections. Such injections were made, without use of anesthetic, by inserting a 26-gauge needle, 2.5 mm long, through the skin and into the holes. Sometimes 2 percent procaine hydrochloride was put on the scalp before injection. For intraventricular injection, a ventricular tube was implanted unilaterally with stereotaxic apparatus (*6*). To make such injections, a 27-gauge needle, long enough to reach the lateral ventricle, was inserted through the tube.

After appropriate taming and food deprivation, preliminary training consisted of 6 days in which the rats were given either a random-reward or a progressive-reward schedule. Drug experiments were run with a random-reward schedule. Twelve trials (48 choices) were given each day. There were 2 days of testing with drug injection, 2 days of testing with saline injection, and then 2 days of testing without injection. This sequence was repeated on the same rats up to five times. Seven micrograms of eserine or DFP, in 0.10 ml of saline, were injected into both visual cortical areas or both somatic areas of each animal on each day of intracortical injection. This dosage was found to be just subconvulsive. For intraventricular injection, three different concentrations of eserine were used (2, 4, and 7 μg in 0.05 ml of saline). In control experiments, the appropriate volume of normal saline was used. Since some fluid may back out from the needle track after injection, the actual amount and distribution of drug in the brain was uncertain (*7*).

No overt behavioral effects were observed in about 30 percent of the drug injections. In the remaining 70 percent of the cases (see Table 1) general behavioral disturbances of various sorts were evident. These ranged from excessive washing, grooming, and chewing to tremor, ataxia, incoordinated walking, and, after intraventricular injection, circling to the side opposite the injection. Finally, grand mal convulsions occurred in 16 cases after drug injection and in six cases after saline injection. The rats usually started to run the maze immediately after injection. When there were severe motor disturbances or convulsions, they remained immovable for periods of from 5 to 15 minutes before running.

The results of maze testing are summarized in Table 1. The number of choices of each rat for each day was calculated, according to the criterion of Krech *et al.*, as visual hypothesis, spatial hypothesis, or no hypothesis (a minimum of 33 out of 48 choices of light or dark was scored as visual hypothesis, of

Table 1. Total number of days on which rats of each strain displayed visual, spatial, or no hypothesis behavior, and general behavioral disturbances under each of the experimental conditions. The number of days of testing of each rat varied from 4 to 30. Every rat of each strain was not run under all the conditions. S, spatial hypothesis; V, visual hypothesis; No, no hypothesis; Beh, general behavioral disturbances; Occ., occipital area; Som., somatic area.

Injection	S_1 (spatial) $N = 13$				S_3 (visual) $N = 13$				S_{13} (mixed) $N = 8$			
	S	V	No	Beh	S	V	No	Beh	S	V	No	Beh
Intracortical												
Eserine, Occ.	7	0	3	5	1	7	4	10	4	2	4	2
Eserine, Som.	7	3	3	10	6	9	5	12	7	0	3	4
DFP, Occ.	6	0	3	6	2	6	1	6	—	—	—	—
DFP, Som.	5	0	2	7	0	7	1	6	—	—	—	—
Saline, Occ.	8	3	3	0	5	9	2	2	7	2	2	0
Saline, Som.	13	0	3	3	4	12	2	1	3	0	3	0
No injection	20	2	2	0	11	27	8	0	3	1	5	0
Intraventricular												
Eserine (2 μg)	7	0	1	1	1	5	2	1	2	4	0	0
Eserine (4 μg)	6	0	2	4	0	8	2	6	2	3	1	2
Eserine (7 μg)	11	3	5	13	2	6	0	5	5	6	4	5
Saline	14	2	4	3	3	15	4	3	4	7	5	1
No injection	25	3	9	0	2	21	6	0	3	12	6	0

left or right as spatial hypothesis). The number of days on which rats of each strain showed any one of the hypotheses was summed under each of the experimental conditions. The results showed no significant differences in hypothesis behavior after drug, or saline, or no injection. There was also no difference in the actual number of choices of the rats. The animals maintained their behavioral pattern whether they were run under drug the *first* time or the *fifth* time, and whether they had had the *random-reward* or *progressive-reward* schedule during the preliminary training. To check the possibility that drug effects might not persist through the entire running period, we used the *t*-test to compare the average scores of the first six trials against the last six trials of each session within each strain of rat under drug injection (only those cases with general behavioral effects were included). The results showed no significant difference. It was striking that the animals, in spite of gross motor difficulties, forced circling, or convulsions between trials, ran the maze according to their usual way. Direct injection of eserine or DFP into the brain of rats affected general behavior in the fashion described, suggesting that such injections had achieved the expected alteration in cortical acetylcholine levels. The fact that such injections did not alter the hypotheses displayed by these animals in running a maze seems to indicate that hypothesis behavior is not dependent on cortical levels of acetylcholine.

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References and Notes

1. D. Krech, M. R. Rosenzweig, E. L. Bennet, B. Krueckel, *Science* 120, 994 (1954); M. R. Rosenzweig, D. Krech, E. L. Bennet, *ibid.* 123, 371 (1956); D. Krech, M. R. Rosenzweig, E. L. Bennet, *J. Comp. and Physiol. Psychol.* 49, 261 (1956).
2. H. McLennan and K. A. C. Elliott, *J. Pharmacol. Exptl. Therap.* 103, 35 (1951).
3. This study was supported by research grants from the National Institute of Neurological Diseases and Blindness [B-801(C1)]; the Fund for Neurobiology, Inc.; and the Wallace C. and Clara M. Abbott Memorial Fund of the University of Chicago. We wish to thank Mrs. Clyde Carrol and Louis Tinnen for their help in some of the experiments.
4. We wish to express our appreciation to Dr. M. R. Rosenzweig and Dr. D. Krech for their kindness in supplying the rats.
5. C. P. Stone, *J. Genet. Psychol.* 35, 559 (1928).
6. We wish to thank Dr. Garth Thomas who made the ventricular tubes and performed the implantations.
7. To help evaluate the consequences of intracortical injection, 12 albino rats were given ten such injections during 2 weeks. Then methylene blue was so injected, and the animals were killed 5, 15, or 30 min later. The dye was spread to about 2 mm around the site of injection after 5 min, but to the entire forebrain at the end of 15 min, mostly in the subarachnoid space and the lateral ventricles. The brains of some experimental animals were serially sectioned and stained with thionin. There was usually a small cavity through the entire thickness of the cortex at the site of intracortical injection. These cavities were lined with glia cells but not encapsulated with connective tissue. In the case of intraventricular injection, besides the needle tract there was sometimes slight damage to structures around the ventricle.

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Concerning a Pigment Commonly Attributed to the Presence of Leuco-Anthocyanin

When an attempt was made to esterify a certain acidic fraction of the water-soluble part of a leaf extract (for example, *Rhododendron ponticum* L.) with boiling 1 percent methanolic hydrochloric acid, the gradual formation of an intense ruby-red coloration was noted. A similar observation had been made previously by O. Rosenheim (*1*), who assumed that a colorless modification of a pigment present in young leaves and stems of the grape (*Vitis vinifera* L.), for which he proposed the general term *leuco-anthocyanin,* was converted by strong acids into what he regarded to be an anthocyanidin. To establish the distribution of the supposed leuco-anthocyan[id]in in the plant kingdom, this method has been applied to leaves of several hundred species of plants (*2*) and to a large variety of other plant materials (*3*). In the majority of cases, the coloration was attributed to the formation of cyanidin and only exceptionally to delphinidin, though no instances are recorded where the anthocyanidin had in fact been isolated or its characteristic absorption curve shown.

In the course of an investigation into the nature of the acidic constituents of leaves, humic acid was isolated (*4*). On heating with 1 percent methanolic hydrochloric acid a pigment was formed, the optical, chromatographic, and chemical behavior of which was identical with that of the pigment obtained when leaves and other plant materials were treated with strong acids. Furthermore, the optical curve of this pigment differs entirely from that of any known anthocyanidin. It shows two characteristic maxima (Fig. 1) in the visible part of the spectrum, one at 548 mμ, $E = 1.76$, the other at 459 mμ, $E = 1.58$ (1 percent methanolic hydrochloric acid) in contrast to the single maximum (Fig. 2) shown by cyanidin at 537 mμ, $E = 1.83$ (1 percent methanolic hydrochloric acid). Also, unlike any anthocyanidin, this pigment is readily decolorized by an alkaline medium such as sodium hydrogen carbonate.

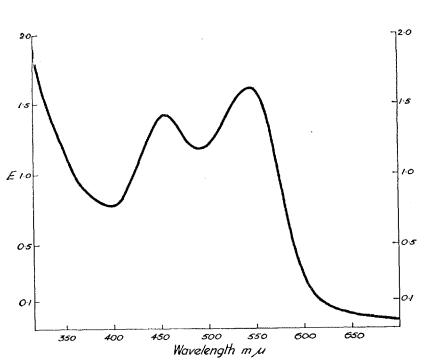

Fig. 1. Optical curve of pigment.

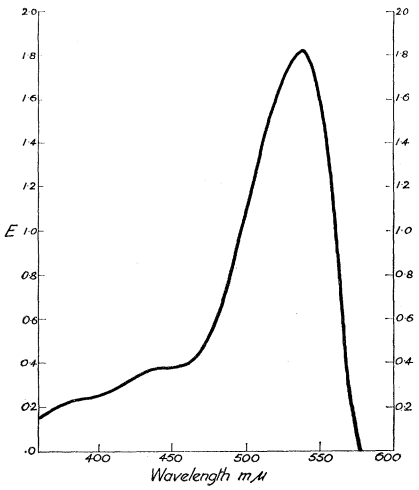

Fig. 2. Optical curve of cyanidin.

So consistent was the formation of this pigment in the case of different plant materials that the color reaction can be considered diagnostic for humic acid, which is almost ubiquitous in the plant kingdom. The chemical and optical properties of the pigment suggest that it is of the trialkyl-methane type and is probably derived from the complicated molecule of humic acid by dehydration and condensation with the aldehyde group present (*5*).

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References and Notes

1. O. Rosenheim, *Biochem. J.* 14, 178 (1920).
2. E. C. Bate-Smith, *ibid.* 58, 122 (1954); ——— and N. H. Lerner, *ibid.* 58, 126 (1954); E. C. Bate-Smith and T. Swain, *Chem. and Ind. (London)* 1953, 377 (1953).
3. For summary of literature, see D. G. Roux, *Nature* 180, 973 (1957).
4. H. Raudnitz, *Chem. and Ind. (London)* 1957, 1650 (1957).
5. I am greatly indebted to Dr. F. B. Strauss, Dyson Perrins Laboratory, Oxford, for the optical data, and to Miss M. Lewis for technical assistance. This work has been financed by the Forestry Commission, to which grateful acknowledgment is made.

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