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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4. We wish to express our appreciation to Dr. M. R. Rosenzweig and Dr. D. Krech for their kindness in supplying the rats.
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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.

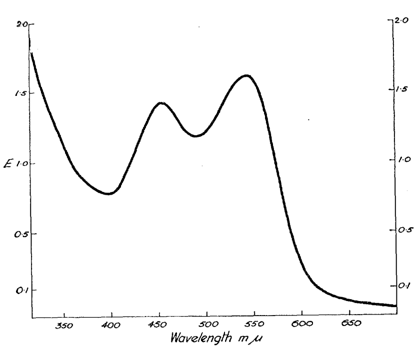

Fig. 1. Optical curve of pigment.

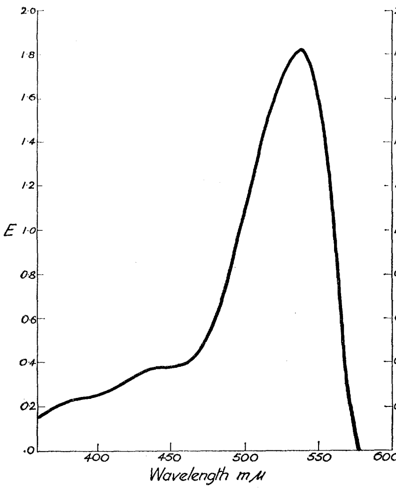

Fig. 2. Optical curve of cyanidin.

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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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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