percent) responded. Receptive adults responded within 2 to 3 minutes after the strips were placed on the cones. The duration of response, while the moths were continuously exposed to the strips, ranged from 2 to 5 minutes. The ability of the male to detect the attractant is lost after exposure and regained only after a "rest" of several hours. The major portion of virgin males initially responded to the presence of the extract when they were 3–4 days old. Initial responses for males whose ages in days were 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, and 9–10 were 5, 20, 29, 26, 10, 6, 2, 1, and 1 percent, respectively. Over 95 percent of all virgin males responded at least once by the time they were 7 days old. A mating response was obtained from one male 17 to 18 days old. Records obtained from frequency-response studies indicated that 80 percent of the virgin males responded from three to eight times to the presence of the extract. The number of males responding 0, 1–2, 3–4, 5–6, 7–8, 9–10, 11–12, and 13–14 times were 1, 10, 23, 32, 47, 14, 6, and 1, respectively. One male responded for 13 consecutive days. Consecutive responses for 2, 3, 4, 5, 6, and 7 days were recorded 68, 36, 15, 17, 9, and 4 times, respectively. The first response of virgin males occurred 1 day after emergence (5 percent) and the maximum response (77 percent) occurred at 8 days. Mortality of males at 8 days was only 8 percent. Thus, in order to insure optimum detection of the attracting substance for a specific period, males at least 7 to 8 days old should be used for bioassay.

The methylene chloride extract of the abdominal tips from 7-day-old virgin females was analyzed by gas-liquid chromatography with a column (2.5 mm × 185 cm), containing 5-percent neopentylglycol succinate on 80- to 100-mesh, acid-washed Chromosorb W, operated at 190°C with a flow of 53 ml of argon through the column per minute. The effluent gases of a very prominent component (or components) having a retention time of 2.6 minutes strongly attracted virgin males and elicited mating responses. The attractant gases emerged approximately midway between the methyl esters of lauric and myristic acid which were used as convenient reference compounds and had retention times of 1.8 and 3.7 minutes, respectively (Fig. 1). The position of the attractant between the two reference compounds indicates that it is fairly volatile and has a relatively low molecular weight. Detectable but lesser quantities of the attractant were found in dead and newly emerged females. No peak was found at the retention time of the attractant component (or components) on chromatographs of methylene chloride extracts prepared from male cabbage looper moth abdominal tips (*6*).

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25 June 1963

Low Dose Radiation of the Developing Brain

Abstract. *X-irradiation administered in single doses of 10 to 40 r has a widespread effect on the developing rat brain. It first diminishes the formation of cytoplasmic basophilic material in the nerve cells and inhibits their growth. Single doses of 20 to 40 r cause permanent alterations of individual nerve cells, and interfere with their organization into neuronal assemblies, such as layers of the cerebral cortex.*

Little work has been done on the pathological effects of single low doses of ionizing radiations on the morphogenesis and cytogenesis of the developing mammalian brain, but related studies (*1*) suggest that low doses of x-radiation may alter neural development. A threshold dose for initiating eye malformations during early neurulation in rats is 25 r and, in mice genetically disposed to show them, certain spinal anomalies are increased when as little as 25 r are given during the formation of the body axis. A threshold dose for killing certain classes of primitive differentiating neural cells in early development in rats and mice is 30 to 40 r. The appearance of four cases of exencephalia in a large series of mice exposed to 5 or 15 r on one of the first 2 days of pregnancy has been attributed to the radiation. In other experiments (*2*), small doses of radiation repeated daily during the fetal life of rats did not lead to cytoarchitectural changes in the adult cerebral cortex when less than 60 r per day were given on five successive days.

We recently began experiments to determine the pathological effects on the developing nervous system of x-irradiation administered in single doses of 10 to 50 r (*3*, *4*). The brains of irradiated rats were studied grossly and histologically, and were compared with matching littermate or cousin controls.

Irradiation with 30 r disturbed the development of the cerebral cortex when given on the 16th, 18th, or 22nd day of pregnancy, or on the day after birth. This disturbance was expressed in several ways depending on which day the radiation was administered. When 30 r was administered on the day after birth, a lag in development of the neurons was seen within 72 hours, particularly of those in the outer half of the cortex, layers 2, 3, 4, and 5. The lag was characterized in the dorsal neocortex by shorter and thinner primary apical dendrites; incomplete delineation and differentiation of layer 4, which is normally under way at this time; and diminished formation and distribution of cytoplasmic basophilic (*5*, *6*) material in the nerve cells, particularly in the more distal parts of the

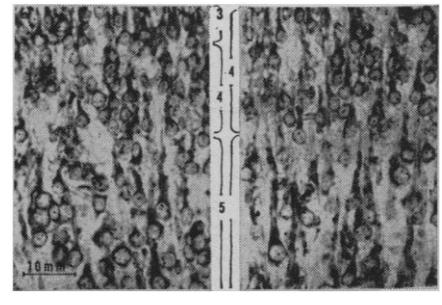

Fig. 1. Diminished growth of neurons in the dorsal cortex (left) of a 4-day-old rat irradiated 72 hours earlier with 30 r, compared with that of a normal litter mate (right). Layer 4 is sparse and cells in layer 5 have smaller, less basophilic dendrites than normal. (× 500, cresyl violet, Luxol fast blue)

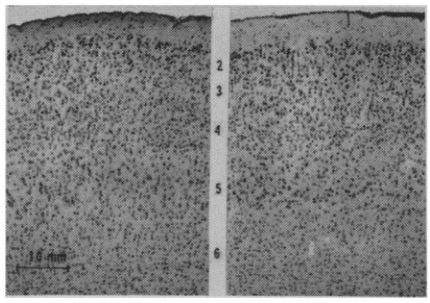

Fig. 2. Indistinct layering of the dorsal neocortex (left) especially layer 4, in a 3½-week-old rat irradiated with 30 r on the day after birth compared with the normal (right). There was an average of 10-percent fewer cells than normal in layers 2 and 3 of this region of the irradiated cortex. The tissue was sectioned at the frontal level of the beginning of the hippocampus. (× 100, cresyl violet, hematoxylin)

dendrites (Fig. 1). There was a general departure of the oganization of the whole cortex from the neat and orderly lining up of neurons which is so characteristic of the normal in these early stages.

Examination of the neocortex after longer invervals following irradiation revealed a poorly delineated layer 4, a general fuzziness of the usual cytoarchitectural arrangement of all the cortical layers, a tendency to clustering of the nerve cells in layer 2, and a sparsity of neurons in layers 2 and 3, often with some shortening of the apical dendrites of the pyramidal cells in those layers. The sparsity can be recognized on inspection; cell counts show the deficit to be 10 to 15 percent (Fig. 2). Glia are neither increased nor decreased in these areas.

A similar train of events was produced by 20 r given on the day after birth, but the consequent deficit in the neurons of the outer layer was less.

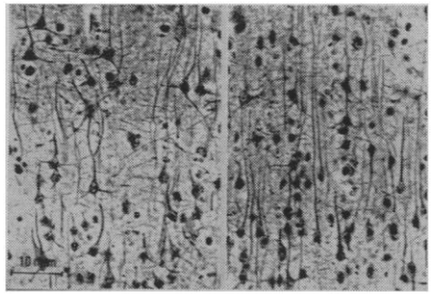

Fig. 3. Disarrangement of neurons and their dendrites in layer 6 of the dorsolateral cortex (left) of 9-week-old rat irradiated with 30 r on the 16th day of intrauterine life, compared with the normal (right). (× 300, Cajal silver pyridine)

The effects of 40 r resembled those of 30 r. To find the lower level of radiation that may alter morphological development of the cortex, we gave 10 r to three members of one litter on the day after birth, and 3 days later we compared their cortexes with those of their four normal litter mates. The lag in development of the dendrites and the changes in their basophilic material were quite similar to those that follow higher doses, but the separation and differentiation of layer 4 from layers 2 and 3 was less affected. The generalized deviation from the normal orderly arrangement of the cortex was, however, just as conspicuous as after 20 to 40 r. We have no data at present on the later consequences of 10 r.

Irradiation with 20, 30, or 40 r on the 16th day of pregnancy led to changes characterized by an irregular arrangement of the neurons, particularly in layer 6 of the neocortex. Normally, the neurons in the frontal section form both vertical and horizontal rows. Their apical dendrites also line up to give an appearance of being vertically straight, a characteristic largely determined by the afferent fibers that enter the cortex from the thalamus and striatum, beginning on the 16th fetal day. After irradiation, we found that the row formation was poor, especially the vertical disposition, and the apical dendrites were less orderly, sometimes wavy (Fig. 3).

Irradiation with 30 r on the 18th day of pregnancy led to a considerable deficit of neurons in the outer cortex. There were about 25-percent fewer neurons in layers 2 and 3 compared with the normal but, as in the brains of animals irradiated on the day after birth, this was not accompanied by a reduction in volume of the neocortex. There was, however, nearly a 50-percent increase in the number of normal glia in the outer parts of the cortex. By way of further comparison, the brain of one animal, from a litter which was exposed to 50 r on the 18th day of intrauterine life, showed a deficiency of outer cortical neurons about equal to that resulting from exposure to 30 r, but clustering of the neurons in layer 2 was evident. Irradiation with 30 r on the 22nd day of pregnancy resulted in changes similar to those following irradiation on the day after birth (based on examination of one member of a litter so treated).

Irradiation with 30 or 40 r on the day after birth resulted in characteristic changes in the cerebellum. The

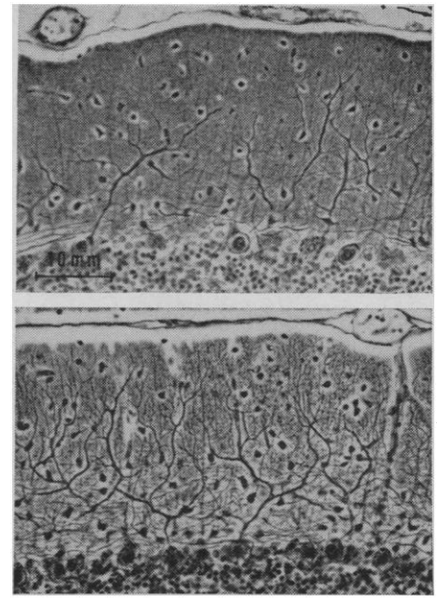

Fig. 4. Irregular dendrites of Purkinje neurons in the cerebellum (above) of 5-week-old rat irradiated with 30 r on the day after birth compared with those of a normal litter mate (below). Cells are in identical regions in lobule 7. (× 150, Cajal silver pyridine)

Purkinje cells, which normally have only the beginning of a single apical dendrite at this time, developed irregular, asymmetrically branching dendrites. This included the Purkinje cells in the folia that comprise lobule 6, which is cytologically the least mature part of the cerebellum on the day after birth. The Purkinje cell in the rat is normally flat and lies in the sagittal plane, reminiscent of a sea fan. The primary dendrite usually bifurcates so that, in sections, this together with the successive branchings form smoothly undulating lines. There may be deviations from this pattern and normal "distortions" may occur at the bottoms of the sulci between folia and over the curves at the crowns of the folia, but in serial sagittal sections of the cerebellum there is a constancy of pattern. Widespread deviations from this pattern were caused, however, by irradiation with 30 or 40 r on the day after birth. Although some cells appeared normal, many showed asymmetrical branching of the major dendrites (Fig. 4). The undulating flow lines were replaced by angular bends, and the fanlike arrangement of the dendrites was often narrowed (*7*).

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3. Methods: Pregnant and newborn hooded rats recently derived from two established lines, an albino and a black non-agouti selfed, were irradiated with x-rays in doses of 10, 20, 30, or 40 r, over the whole body, in lucite containers ⅛ in. thick, on the 16th, 18th, or 22nd day of pregnancy or on one of the first 3 days after birth. In addition, one litter was irradiated on the 18th day of pregnancy with 50 r and members of another litter were given 47 r on the day after birth. The irradiated offspring were killed at intervals of 24 hours, 72 hours, 1, 2, 3½, 5, 9, or 10 weeks later with precisely matched litter mate or cousin controls. We irradiated 137 animals during development, and there were 83 controls. Of these, 41 animals, irradiated on the 16th, 18th, or 22nd day of pregnancy or on the day after birth, were drawn as samples for histologic study with 31 of the controls. Ten of the 41 animals had received 20, 30, or 40 r on the day after birth, and these were compared 72 hours later with 12 litter mate controls. Another 8 animals from the same litters were compared with 10 matching controls at the other intervals up to 9 weeks. The forebrain from just in front of the anterior commissure back to the level of the habenular nucleus was sectioned serially either in paraffin at 8 μ or in frozen sections at 20 μ. Serial sections were made sagitally of one sagittal half of the cerebellum with attached brain stem, usually in frozen sections at 20 μ, but paraffin was used in four early-stage animals. In some animals both the forebrain and cerebellum were studied. Matching paraffin sections stained with cresyl violet and Luxol fast blue, or frozen sections stained with Cajal pyridine silver were compared directly on viewscopes, by direct observation of superimposed sections, and by photography. Pencil tracings of projected images of the dendrites of groups of corresponding normal and abnormal Purkinje cells were also compared. The radiation factors were Westinghouse Coronado therapy unit, 250 kv at constant potential, 9 ma, no added filter, 70 cm from source to middle of animal's body, 59.7 r per minute. The radiation was measured by cylindrical wax phantoms equivalent to the tissue and corresponding in size to the pregnant and newborn rats in their experimental surroundings.

   Cell counts were made with a ruled square eyepiece reticule, the magnification being adjusted to cover layers 2 and 3 without encroaching on layer 4, whose fuzzy outline in the one-day old irradiated animals made numerical comparisons of this layer difficult. For example, the number of neurons in three alternate columns of the seven columns in the reticule was counted in the dorsal and dorsolateral cortex in five to ten successive paraffin sections. The same thing was done in the precisely corresponding areas of the cortex to be matched. Several parts of different cortical regions were counted. At the magnification most often used (× 160), the reticule covered an area of 0.15 mm² and enclosed about 250 neurons in layers 2 and 3 of the dorsal cortex of a 3½-week-old normal rat.
4. We thank Dr. Charles S. Simons, department of radiology, for carrying out the radiation dosimetry.
5. The basophilic material of the cytoplasm has been described previously [S. P. Hicks, M. C. Cavanaugh, E. D. O'Brien, *Am. J. Pathol.* **40**, 615 (1962)] as being present diffusely in the cell body and the early apical dendrite when the primitive neuron first begins to differentiate under normal conditions. With subsequent postnatal growth and differentiation, it becomes progressively concentrated in special places: (i) at the bases of spurs which give the first evidence of dendrite branches along the main dendrites; (ii) in minute aggregates along the neurofibrils of the young main dendrites; and (iii) somewhat diffusely in the cell body cytoplasm up to the stage when aggregated Nissl substance is formed. This basophilic material absorbs ultraviolet light of 2630 angstroms and is removable by ribonuclease. It is considered by us and others (*6*) to be an indicator of cytoplasmic RNA. In the day-old rat, the cells of 2, 3, and 4 are the most immature members of the cortex, with simple, early, diffusely basophilic apical dendrites; in layers 5 and 6, which are more mature, the cytoplasmic basophilic material has begun to be distributed in the special places just mentioned. In the present experiments, we found that radiation on the day after birth retarded the normal progression of this distribution and, at the same time, the dendrite growth that parallels it was retarded. Although these first morphological changes following irradiation resembled those that follow anoxia of day-old rats, the consequences in later life were quite different.
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17 June 1963

Flavin Sensitized Photoreactions: Effects of 3-(p-Chlorophenyl)-1,1-Dimethylurea

Abstract. *Photooxidation reactions, which were inhibited by high concentrations of oxygen, were affected by the addition of 3-(p-chlorophenyl)-1,1-dimethylurea (CMU), but the response varied with the substrate being oxidized. With ascorbic acid and 2,3-diketogulonic acid, CMU reversed the inhibition caused by high concentrations of oxygen. With ethylenediaminetetraacetic acid (EDTA), tetraethylene tetramine, or Mn^{++} as reductant, CMU itself inhibited the reactions. The photoreduction of flavins by EDTA and the bleaching of flavin mononucleotide under anaerobic conditions were also inhibited by CMU. Corresponding photoreactions sensitized by phthaleine dyes or methylene blue were completely insensitive to CMU. This compound therefore seems to change specifically the reactivity of excited flavin molecules.*

The stoichiometric formation of the end products of photosynthesis and the high efficiency of photochemical reactions in living cells are achieved with the aid of the complex molecular structure in which the sensitizing pigments are imbedded. In homogeneous solution, by contrast, the existence of alternate chemical pathways for seemingly simple photoreactions has frustrated many workers in their attempts to establish a clear-cut description of the process.

Habermann and Gaffron (*1*) observed that the flavin-sensitized photooxidation of ascorbic acid, in the presence of catalase and Mn^{++}, proceeds in two steps. When the amount of oxygen taken up during the first step approaches one equivalent, the rate of oxidation becomes very slow. Then the photooxidation suddenly starts again and a second equivalent of oxygen is consumed. Thus, with only a slight increase in the complexity of a mixture of reagents in solution, a pronounced order is established in the succession of photoreactions induced by a sensitizing dye. We have now studied how certain photooxidations are influenced by 3-(*p*-chlorophenyl)-1,1-dimethylurea (CMU), which is known to be a highly specific inhibitor of photosynthesis (*2*).

In the absence of catalase, the flavin-sensitized photooxidation of ascorbic acid has been reported to lead to an ascorbic acid hydroperoxide which slowly decomposes to threonic acid lactone and oxalic acid (*3*). It appears that, in the first step, the catalase decomposes the ascorbic acid hydroperoxide yielding dehydroascorbic acid, which, under the experimental conditions, hydrolyzes to 2, 3-diketogulonic acid. It is this latter compound which is photooxidized in the second step, provided Mn^{++} is present, to oxalic acid and threonic acid; but this oxidation does not start until all the ascorbic acid is used up. Such inhibitions of photochemical reactions by small amounts of ascorbic acid have been noted before (*4*). We observed that the time required for the oxidation of the last traces of ascorbic acid at the end of the first step could be markedly lengthened by increasing the oxygen concentration (see Fig. 1.) At low oxygen concentrations, the rate of oxidation of ascorbic acid remained almost constant. The rate of the second oxidation also decreased with increasing concentrations of oxygen. Similar inhibitive effects of oxygen on photooxidations have been reported by Bäckström and other investigators (*5*). We found that iodide also inhibits both steps in the photooxidation of ascorbic acid.

Addition of CMU had the effect of reversing the inhibition by iodide, or completely abolishing the inhibition by oxygen (Fig. 1). Thus, when the concentration of oxygen was 70 percent, and when CMU was present, the course of the two-step reaction was almost the