last 2 yr (*2, 3*). Furthermore, it has been demonstrated that removal of either adrenal glands or spleen from the nonirradiated partner does not significantly alter the postirradiation protection afforded by parabiosis (*4*). In view of these findings it seemed pertinent to determine the influence of certain endocrine glands on irradiation protection by pairing irradiated rats with nonirradiated, hypophysectomized partners. If a parabiont without a pituitary gland were still capable of protecting its partner from irradiation death, much speculation concerning the role of the endocrine system in irradiation protection might be eliminated.

Holtzmann female littermate rats were used throughout this investigation and experimental procedures were carried out when the animals were about 40 days of age and had attained a weight of 120 ± 5 g. The parapharyngeal approach without cannulation of the trachea was used for hypophysectomy and the operation usually preceded the pairing by 2–3 days. All rats to be irradiated were placed in plastic boxes and exposed to ionizing radiation with the following physical factors: 250 KV, 30 ma, 0.25 Cu and 1.0 Al filtration, HVL 0.88 Cu, 50 cm FSD, 20 × 20 cm field, and an intensity of 127 r/min. The animals were exposed to a dose of 700 r which previously had been determined as the LD 98/30 days in this laboratory. The animals were divided into 4 groups and all consisted of parabionts except the first which was composed of 65 single irradiated controls. Parabiosis was carried out under Nembutal anesthesia, according to the method of Bunster and Meyer (*5*), within 3 hr after exposure to x-ray. Unless death intervened, animals were observed for a 30-day period, at which time surgical separation of the pairs was done under ether anesthesia. At the same time the hypophysectomized partners were sacrificed in order to determine completeness of the operation. Sella turcicas were checked grossly and microscopically.

Results are summarized in Table 1. Of the 65 single animals exposed to 700 r, only one survived the 30 day observation period and that one succumbed on the 75th postirradiation day. Twenty-three nonirradiated–nonirradiated pairs made up the 2nd group and of these 16 survived 30 days for a survival rate of 70%. A small group of irradiated–irradiated parabionts served as further controls for parabiosis and as was expected, mortality of these pairs was complete. In most cases deaths occurred somewhat earlier than in the single irradiated animals, presumably due to the added stress of the operation. Irradiated-hypophysectomized pairs make up the last group and of the original 22 pairs, 16, or 73%, survived 30 days or longer. Only those pairs in which the hypophysectomized partner showed no remnants of the hypophysis have been included. A significant difference exists between survival in the unpaired irradiated control group and survival in the group of irradiated animals with hypophysectomized partners ($P < 0.01$).

The effects of parabiosis are shown in the irradiated-irradiated and nonirradiated-nonirradiated parabionts. In the former group death was considered to be only a result of irradiation since it occurred within the first 5 or 6 days. Deaths in the latter group, which was not irradiated, were attributed primarily to parabiosis intoxication, a condition peculiar to parabiotic animals (*6*). In the irradiated-hypophysectomized group both factors were responsible for the mortality.

TABLE 1

Treatment*	No. of pairs	Survival at 30 days following irradiation
Unpaired irradiated	65	1 (2%)
Nonirradiated–nonirradiated	23	16 (70%)†
Irradiated–irradiated	10	0
Irradiated-hypophysectomized	22	16 (73%)†

* All irradiated animals received 700 r.
† Statistically significant difference from unpaired irradiated rats.

This experiment represents one of a series in which the mechanism of postirradiation protection by parabiosis is being sought. Previous work has shown that neither the adrenals nor the spleen are necessary in the nonirradiated partner to produce the protection. Now, good protection has been described when irradiated rats were paired with nonirradiated, hypophysectomized animals. Work of this nature tends to eliminate the role of the hypophysis and its dependent endocrine organs as critical factors in recovery from irradiation sickness.

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Accumulation of Acid-Soluble Nitrogen in the Brain Cortex of Cats During Stimulation

A. Geiger,[1] J. Dobkin, and J. Magnes[2]

Department of Physiology, The Hebrew University-Hadassah Medical School, Jerusalem

In the experiments described below it is shown that trichloracetic filtrates of brain cortex frozen during stimulation contain considerably more nitrogenous compounds than those of homologous areas taken at rest.

Small increases in the concentration of ammonia in the brain during stimulation have been described by

[1] Present address: University of Illinois, College of Medicine, Neuropsychiatric Institute, 912 S. Wood St., Chicago, Ill.
[2] The authors wish to express their appreciation to M. Samueloff for his help with the experiments.

TABLE 1

INCREASE OF NPN IN THE BRAIN DURING STIMULATION

Expt.	Cortical area	NPN mg % in TCA filtrate: Non-stimulated hemisphere frozen 2 sec before stimulation	NPN mg % in TCA filtrate: Hemisphere stimulated through contralateral brachial plexus	Duration of stimulation, sec
1	Frontal	164	167	7
	Central	174	193	
	Occipital	184	189	
2	Frontal	130	138	7
	Central	150	168	
	Occipital	153	164	
3	Frontal	91	108	20
	Central	149	178	
	Occipital	180	185	
4	Frontal	108	164	15
	Central	142	168	
	Occipital	149	163	
5	Frontal	155	182	15
	Central	170	202	
	Occipital	187	187	
6	Frontal	140	175	15
	Central	152	205	
	Occipital	173	193	
7	Whole frontal and central	106	152	15
Demonstration of reversibility: both hemispheres frozen 40 sec after stimulation				
1	Frontal	125	115	15
	Central	132	124	
	Occipital	178	178	
2	Frontal	102	100	15
	Central	148	151	
	Occipital	181	177	

previous investigators (*1*); these changes, however, were of the order of less than 1 mg % N.

In the present experiments the brain cortex in Nembutal narcosis was stimulated either through directly applied electrodes, or through the contralateral brachial plexus with ac of 40 v and 75 c, after the removal of the skull over a wide area. The nonstimulated hemisphere was frozen 2 sec before starting stimulation, by applying a metal container filled with solid CO_2 and acetone. Freezing was instantaneous, and the temperature of the opposite hemisphere remained unaffected 7–8 sec. After a period of stimulation lasting 7–15 sec, the other hemisphere was also frozen and the frozen areas were cut out and divided into frontal, central, and occipital parts. Most of the white matter was sliced off, leaving slices about 2.5 mm thick, consisting mainly of gray matter, which were homogenized in their frozen state in 10% trichloracetic acid.

In the filtrates, total N was determined by Nesslerization after digestion with sulfuric acid and potassium persulfate. Amino-N was estimated by the method of Moore and Stein (*2*). Volatile bases were liberated and estimated, according to Dobkin (*3*), by treating the neutralized TCA filtrates with alkaline potassium permanganate in Conway dishes at 38° C overnight and titrating the mixture of bases collected in the acid compartment. From similarly treated duplicates, the contents of the central compartment were transferred into the outer chamber of another Conway dish, and, after the addition of formaldehyde and alkali, the trimethylamine present in the mixture was distilled into standard acid and titrated. Choline was calculated on the basis of the trimethylamine thus found.

In a large number of preliminary experiments, it was ascertained that the nonprotein nitrogen concentration in homologous areas of both hemispheres was practically identical at rest. In different cortical areas of the same hemisphere, however, the nonprotein N concentrations varied, being lowest in the frontal and highest in the occipital areas.

The results obtained by direct stimulation of the cortex were identical with those obtained when the brachial plexus was stimulated. In the experiments presented in Table 1, the cortex was stimulated through the brachial plexus.

The changes in the NPN content of the cortex during stimulation were very marked, mainly in the central and frontal areas. In the latter, the average increase of NPN during stimulation was around 25 mg % N. In some experiments, 40–60 sec after discontinuing stimulation the differences between stimulated and resting areas had disappeared. In most reversion occurred only after the lapse of 10–20 min.

The amino-N concentration was lower in the directly stimulated areas than in the corresponding resting areas, as shown in Table 2. These differences were less marked when the brachial plexus was stimulated.

In the majority of experiments, higher amounts of volatile bases were released by alkaline permanganate treatment from the TCA filtrates of the stimulated cortex than from corresponding resting areas of the opposite hemisphere. The amount of volatile bases found during stimulation was usually very close to 35 meq/kg. There was no increase in the concentra-

TABLE 2

EFFECT OF DIRECT STIMULATION OF THE BRAIN CORTEX ON NH_2-NITROGEN, AS MEASURED BY MOORE AND STEIN NINHYDRIN COLORIMETRIC METHOD

Date	Area	NH_2-nitrogen mg %: Non-stimulated	NH_2-nitrogen mg %: Stimulated
10/3	Central	52	46.0
10/3	Central	43	39.5
10/3	Central	55	41.0
16/3	Central	43	39.0
19/3	Central	47	38.5
23/3	Central	46	42.5
7/4	Central	45	41.0
	Frontal	78	54.0
4/5	Central	63	46.4
	Occipital	56	42.6

TABLE 3

VOLATILE BASES RELEASED FROM TCA EXTRACT OF BRAIN CORTEX BY OXIDATION WITH ALKALINE $KMnO_4$

Expt.	Area	N mg % non-stimulated	Stimulated
1	Frontal and central	23.0	32.0
2	Frontal and central	22.0	43.0
3	Frontal	31.5	32.5
	Central	33.0	43.0
	Occipital	39.5	43.5
4	Frontal	39.5	45.0
	Central	45.0	45.0
	Occipital	44.5	47.5
5	Frontal and central	36.8	50.0

tion of these bases during stimulation in spite of a considerable increase of NPN, when their resting concentration reached this figure.

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Effect of Phosphorylated Hesperidin and Other Flavonoids on Fertility in Mice

R. Q. Thompson, M. Sturtevant, and O. D. Bird

Research Laboratories,
Parke, Davis and Company, Detroit, Michigan

Several reports have appeared recently concerning the antifertility activity of phosphorylated hesperidin, a compound previously shown (*1*) to be a hyaluronidase inhibitor. This derivative of hesperidin has been reported to prevent conception in rats (*2*), mice, and humans (*3*) when administered orally or intraperitoneally. However, Chang and Pincus (*4*) were unable to confirm the reported activity of phosphorylated hesperidin, and furthermore indicated that a hyaluronidase inhibitor would not necessarily be an inhibitor of fertilization. Subsequently Martin (*5*) reported that the phosphorylated hesperidin as originally used was a mixture of phosphates, only one of which acts as an antifertility agent.[1]

In the present investigation, initiated before publication of the reports by Chang and Pincus (*4*) and Martin (*5*), three flavonoids, phosphorylated hesperidin,[2] dihydroquercetin, and hesperidin methyl chalcone were tested for antifertility activity in mice. Rodney *et al.* (*6*) have shown that dihydroquercetin inhibits hyaluronidase *in vivo* to a greater extent than most of the other flavonoids tested by these investigators. Although hesperidin methyl chalcone does not inhibit hyaluronidase (*6, 7*), it seemed of interest to include this compound in the antifertility tests because of its similarity in structure with the two other flavonoids being tested.

[1] Following the completion of this paper for publication, Millman and Rosen (SCIENCE, **118**, 212 [1953]) reported that phosphorylated hesperidin did not reduce fertility in mice or rats treated orally or by intraperitoneal injection.

[2] We wish to thank G. J. Martin, of the National Drug Co., Philadelphia, for supplying the phosphorylated hesperidin.

TABLE 1

PREGNANCY IN MICE

Group No.	Compound*	No. males	No. females	No. pregnant	Gestation period† days
1–6	None	6	24	22	21–34
7, 8	Phosphorylated hesperidin	2	8	8	21–30
9, 10	Dihydroquercetin	2	8	6	21–31
11, 12	Hesperidin methyl chalcone	2	8	8	21–23

* Each compound incorporated in diet at a level of 0.875 g/kg.

† Refers to number of days from the time male was introduced to the female to the day of parturition.

Weanling mice were separated according to sex and maintained on a laboratory breeding ration consisting of ground grains, alfalfa leaf meal, brewers' yeast, and whole liver powder until the mice were 7 wk old. At this time 3 females were placed with each male for fertilization. Pregnancy records were kept for each group. Following the parturition of their first litter, 48 fertile females were arranged in 12 groups of 4 each. In addition, 12 males of proved fertility were housed individually. Each of the 3 flavonoids to be tested for antifertility activity was incorporated at a level of 0.875 g/kg of the breeding ration, respectively. Since 5–6 g of diet were consumed/day/mouse (weighing 25–35 g), the level of flavonoid used afforded an intake of approximately 150 mg/day/kg of body weight. Two groups of females and 2 males were maintained on the respective diets 10–12 days prior to placing the males with the females. Each group continued to receive their respective diets during mating. The remaining 6 groups of females and males received the unsupplemented breeding ration and served as controls. About 2 wk following the introduction of the male, the females were examined daily for pregnancy and removed from the male if pregnant. The data obtained are presented in Table 1. The results of this investigation indicate that fertility in mice was not affected by any of the compounds tested under the conditions of this study.

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