weight, corrected for buffer) was made up to 1.0 ml with additions of 102 μg indole (0.87 micromoles) and 2 mg DL-serine, and incubated at 37° C. Good agreement was obtained between indole disappearance and tryptophan formation utilizing Ehrlich's reagent essentially as described (*3, 7*).

The considerably lessened activity of the zinc-deficient enzyme preparations could not be obtained with separate deficiencies of iron or manganese (Table 1). Lack of inhibitory effects resulting from intermixing experiments employing zinc-deficient and control extracts ruled out the possibility of the presence of an inhibitor in the zinc-deficient preparations. The addition of Zn^{++} (ranging from 2×10^{-6}M to 5×10^{-2}M in final concentrations) to the cell-free extracts of zinc-deficient material failed to restore enzyme activity. At concentrations of 2×10^{-4}M and 10^{-3}M, Zn^{++} actually caused a 66% and a 97% inhibition, respectively, in the controls. Exposure of zinc-deficient mats under sterile conditions to Zn^{++} at a level of 2 μg/ml (as in Fries basal medium) in a nitrogen-free medium did not enhance enzyme activity after 53 hr. Pyridoxal phosphate,[2] which has been shown to be active in this particular enzyme system (*6*), only slightly improved the synthesizing power of the zinc-deficient extract. Separate additions of Cu^{++}, Mg^{++}, Mn^{++}, and acid-hydrolyzed casein to the zinc-deficient extracts failed to restore enzyme activity. Cu^{++} was inhibitory to the control extracts at low concentrations, and Mg^{++} showed toxicity at higher values. Cysteine was ineffective in reactivating the zinc-deficient preparations and, at a final concentration of 3×10^{-2}M, caused a 59% inhibition in the activity of the control preparations. The metal binding agents, 8-hydroxy quinoline and ethylenediamine tetraacetic acid, proved to be ineffective in the control extracts as inhibitors except at high concentrations (0.3% and 2%, respectively). Potassium cyanide at final concentrations of 10^{-2}M and 10^{-1}M caused a 46% and a 92% inhibition, respectively, in the control preparations. Potassium thiocyanate gave 83% inhibition at 10^{-1}M.

TABLE 1

TRYPTOPHAN SYNTHESIS FROM INDOLE AND SERINE BY CELL-FREE ENZYME EXTRACTS FROM 5-DAY-OLD *Neurospora crassa* GROWN ON CONTROL MEDIA AND MICRONUTRIENT ELEMENT-DEFICIENT MEDIA

Cell-free extract	Indole lost	Tryptophan formed	Indole lost	Tryptophan formed	Indole lost	Tryptophan formed
	after 1 hr micromoles × 10^2		after 2 hr micromoles × 10^2		after 3 hr micromoles × 10^2	
Control mats						
Exp. 1	43	44	83	82	83	83
" 2	39	49	84	92	91	88
" 3	51	53	88	79	—	—
" 4 (3-day-old mats)	40	40	81	79	90	83
Average Zinc-deficient mats	43	47	84	83	88	85
Exp. 1	3	8	8	10	18	19
" 2	6	10	16	19	22	24
" 3	0	5	0	2	—	—
" 4 (3-day-old mats)	19	16	34	27	32	35
Average Iron-deficient mats	7	10	15	15	24	26
Manganese-deficient mats	31	35	65	66	84	94
	32	28	76	76	85	82

It would appear from these data that a relationship exists between zinc and the enzyme which converts indole and serine to tryptophan. It remains to be determined whether zinc is an actual constituent of the tryptophan-forming enzyme or whether it is concerned, directly or indirectly, in the synthesis of one or more constituents of the enzyme system.

Recent experiments in this laboratory also indicate that zinc deficiency leads to alterations in the activity (either increase or decrease) of certain enzymes, whereas other enzymes are not affected (*1*).

[2] Kindly supplied by I. C. Gunsalus.

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Vitamin P Protection against Radiation[1]

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Griffith *et al.* were the first to demonstrate the protective action of flavonoids in radiation injury (*2*). Clark and associates found that "a flavonoid preparation derived from lemons, administered in the drinking water to guinea pigs, reduces the mortality from total-body roentgen irradiation by about half" (*1*). Field and Rekers conducted an extensive investigation on the protective action of vitamin P factors, using dogs. Mortality after radiation was reduced to 10%–17% (with a significant reduction in the hemorrhagic diathesis) as against 60% mortality in the control animals. The investigators concluded "that previous misunderstanding of the nature of vitamin P has arisen from both the failure to recognize that several flavonone analogues pos-

[1] Condensation of paper presented at the annual meeting of the Florida Academy of Sciences, December 3, 1949, at Stetson University.

[2] Carll Tucker Fellow.

TABLE 1

CONTROL GROUP OF 20 IRRADIATED RATS*

No. of days of survival	11	12	13	14	15	16	17	18	19	20	21	22	23
No. of rats that succumbed	1	2	2	1	3	0	3	2	1	0	0	0	1

* These rats did not receive vitamin P.

sess very similar antihemorrhagic activity and that ascorbic acid has the capacity to potentiate activity in other flavonones'' (*3*, *4*).

In our investigation, 50 rats of British brown breed (obtained from Francis Carter Wood of St. Lukes Hospital, New York City) were submitted to x-ray irradiation. One group of 20 rats served as control, and a second group of rats was given vitamin P compound (CVP compound) isolated from citrus waste.[3] The average weight of the rats was 180 g, ranging from 160 to 205 g. The rats were kept on regular Purina Rat Ration. The radiation factors were 250 kv, 15 ma, with 0.5-mm Cu and 3.0-mm Bakelite filters. Target distance was 27.5 cm, and 210 r/min was the dose rate. All rats received 800 r total-body radiation in a single exposure.

Sixteen rats of the control group (80%) succumbed during the second and third weeks after the exposure (Table 1). All of them manifested gross hemorrhages of various gravity and pronounced pathological lesions in the adrenal glands. The zona fasciculata and zona reticularis were particularly affected, with argentaffin fibrils showing signs of degeneration. Four rats (20%) survived in spite of numerous petechial hemorrhages and generalized purpura.

TABLE 2

IRRADIATED RATS GIVEN 40 MG OF VITAMIN P

No. of days of survival	18	19	20	21	22	23	24
No. of rats that succumbed	1	0	0	0	1	1	1

The treated animals were divided into two groups. Ten rats received orally 4 mg of vitamin P compound per day for 10 days, 3 days prior to radiation and 7 days after radiation. Twenty rats received 5 mg of vitamin P per day for 30 days, 7 days prior to radiation and 23 days after radiation.

In the group of animals (Table 2) which received a total amount of 40 mg of vitamin P compound, the mortality from irradiation was reduced to 40%. Moreover, those rats which did not succumb to the injurious effect of radiation lived longer. The petechial hemorrhages in the treated animals were considerably less pronounced, but some pathological changes in the adrenal cortex were observed, mostly in the zona reticularis (vacuolization).

In the group given a total of 150 mg of vitamin P compound in a period of 30 days, mortality from irridiation was reduced to 10% (Table 3). In this group, petechial hemorrhages were very slight and in some rats apparently absent.

[3] This compound, containing four identified factors naturally present in citrus fruit, was obtained from Vitamerican Company, Paterson, New Jersey.

TABLE 3

IRRADIATED RATS GIVEN 150 MG OF VITAMIN P

No. of days of survival	18	19	20	21	22	23	24	25
No. of rats that succumbed	1	0	0	0	0	0	0	1

From these observations it appears that the vitamin P compound, which contains four flavonoids naturally present in citrus fruit, gives considerable protection to rats against a total-body, near-lethal dose of radiation.

In our previous publication (*5*), we stressed the importance of making a clear distinction between increased capillary permeability and capillary fragility. In radiation injury, there seems to be present a pronounced increase in capillary fragility which might be prevented by large doses of flavonoids naturally present in citrus fruit.

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Likelihood of Photorespiration or Light-inhibited Respiration in Green Plants[1]

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Generally speaking, there are two ways in which light could stimulate respiration: either directly by photosensitization or indirectly through photosynthesis, thereby increasing the amount of respirable metabolites. It is the direct effect of light on respiration (photorespiration), commencing upon exposure to light and stopping immediately upon the return to darkness, that has been a source of disturbance to those interested in measuring photosynthesis, as such a process escapes measurement. Whenever light has been reported to stimulate respiration, the stimulation has been of the persistent or indirect type and could be explained either on the basis of an accumulation of photosynthates, or on the basis of light absorption by the carotenoids, in which case respiration has been found to increase slowly in the light and persist for a short time in the dark. The possibility also

[1] This experiment was part of a dissertation presented in 1949 in partial fulfillment of the degree of Doctor of Philosophy in Yale University.

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