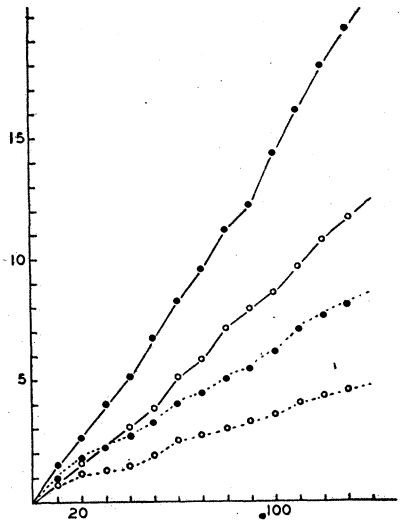

FIG. 1. Oxygen uptake of active and blocked intact embryos and homogenates; ordinates, mm³ O_2/100 embryos or equivalent; abscissas, time in min. Solid circles represent active embryos; open circles, blocked embryos; solid lines, intact embryos; broken lines, homogenates.

Results from many experiments seem similar, and in Fig. 1 are shown graphically typical curves for morphologically similar embryos and homogenates made from them. During diapause or block, oxygen uptake is always much lower than for active, nonblocked embryos. As a matter of fact, one judges the degree of block by the extent to which oxygen uptake is lowered in comparison with that of actively developing embryos. An inspection of Fig. 1 shows that oxygen uptake of intact embryos is always higher than that of the homogenates made from them. Oxygen uptake of homogenates for blocked embryos is correspondingly lower than that for homogenates of the active ones. The relative difference in oxygen uptake of embryos and homogenates, both for the active and blocked conditions, is surprisingly constant and has been found consistent in all experiments thus far carried out. Approximately 65% of the total respiration in both active and blocked cells is due to what one might term "physical structure or intactness," whereas approximately 35% is due to the basic chemical components of the system. A striking point is that a more or less linear relation between oxygen uptake and time for both intact embryos and homogenates is found (see Fig. 1). Dilution of the homogenates up to twenty times gives a straight line over a 2-hr period, showing that oxygen uptake is proportional to cellular concentration (Fig. 2).

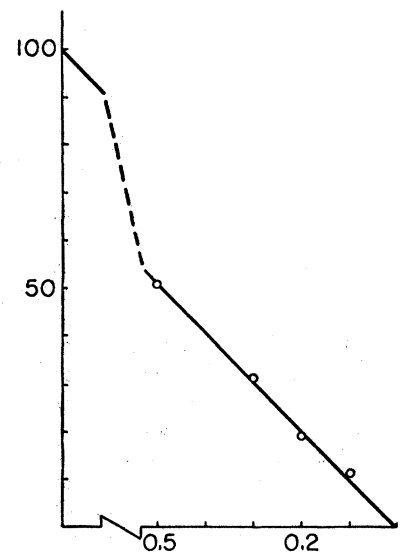

FIG. 2. Effect of dilution on oxygen uptake rate of homogenates, using original concentration (100 embryos/ml) as 100%; ordinates, % of normal oxygen uptake rate; abscissas, dilution of original homogenate.

Inasmuch as homogenates of active and blocked embryos show characteristic oxygen uptake, such systems when further analyzed should show physicochemical differences in the two physiological states of the cell. Further data on these points will be presented elsewhere.

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Effect of Zinc Deficiency on the Synthesis of Tryptophan by *Neurospora* Extracts[1]

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It has been reported that zinc is required directly for the synthesis of tryptophan and indirectly for the synthesis of auxins in tomato plants (*2*, *5*). It has also been demonstrated that tryptophan can be synthesized from indole and serine by wild-type *Neurospora* (*4*), as well as by cell-free extracts of the same organism (*6*). The present study with *Neurospora* indicates that cell-free extracts of zinc-deficient mycelia affect unfavorably the formation of tryptophan from indole and serine.

Cell-free enzyme extracts were prepared from the mats of *Neurospora crassa* (5297A) grown for 5 days in Fries basal medium, and from those grown on the same medium lacking zinc. The growth of the latter was $\frac{1}{3}$–$\frac{1}{2}$ that of the controls. The mycelial mats were washed with triple-distilled water, frozen, homogenized in three times their weight of 0.1M phosphate buffer at pH 7.5 and centrifuged. Five-tenths ml of the supernatant (10 mg dry

[1] Contribution No. 2 of The McCollum-Pratt Institute.

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.