added to the enzyme-coenzyme complex. The formation of each of these complexes results in changes in the activation spectrum similar to those shown in Fig. 1 for the LDH-DPNH-L-lactate complex (*9*).

With the advent of commercial spectrophotofluorometers by means of which either activation or fluorescence spectra can be recorded, a powerful tool for the investigation of enzyme-coenzyme interactions is available. Since previous attention has been limited to emission spectra, it seemed desirable to call attention to the usefulness of activation spectra for these studies.

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28 April 1958

Root Curvatures Induced by Culture Filtrates of Aspergillus niger

Abstract. Evidence obtained by paper chromatography indicates that corn root curvatures caused by culture filtrates of *Aspergillus niger* are caused by the same compound which causes curvatures and malformations on the stems and petioles of bean plants. The R_f values obtained for this compound were substantially different from those of indoleacetic acid.

I recently reported that when culture filtrates of the fungus, *Aspergillus niger,* are used to treat the growing points of bean seedlings, severe curvatures and malformations are produced on the subsequent growth of the plants (*1*). Malformations consisted of greatly thickened stems and petioles, tumorlike stem protrusions, severely twisted stems, and stems enlarged in only one plane to produce a stem that was wide and relatively flat. Most frequently, curvatures consisted of strong downward bendings of the elongating stem and the compound leaves. In addition, elongation of the first and second internodes above the primary leaves was inhibited. Little or no effect was noted when corn seedlings were treated with the culture filtrate. This report concerns the induction of root curvatures by culture filtrates of *A. niger.*

The methods used for growing the fungus on corn steep–glucose medium and obtaining the culture filtrates were described in the earlier report (*1*). Culture filtrates (*p*H 5) were extracted three times with equal volumes of ether; the ether was removed by evaporation, and the residue was brought up in water and diluted to varying concentrations. Approximately 2.5 ml of the solutions was used to moisten Whatman No. 1 filter paper (9.0 cm) which had been previously autoclaved in petri dishes. Corn seeds (the single cross WF9 × 38-11) were washed thoroughly in deionized water, and six seeds were placed in each petri dish on the periphery of the filter paper. The seeds were arranged in sets of three on opposite "sides" of the dish and oriented so that the roots would grow across the dish toward one another. The seeds were incubated at 27°C and examined at the end of 72 hours.

Figure 1 illustrates the curvature of the roots when the seeds were germinated on the *A. niger* extract (bottom) as compared with seeds placed on water (top) or on an ether extract of the uninoculated culture medium (middle). In a number of cases the roots on the *A. niger* extract formed several complete circles in a tight coil to give the appearance of a corkscrew. Although no quantitative experiments have been performed, it has appeared that the best concentrations for producing root curvatures are between 1/20 and 1/50 of the normal concentration of the culture filtrate. In several experiments, no curvatures were obtained when the seeds were placed on the *A. niger* extract at a concentration equal to that of the unextracted filtrate. At concentrations ranging from 1/20 to 1/50 of that of the unextracted culture filtrate, 50 to 100 percent of the germinated seeds showed strong root curvatures.

It remained to be shown that the compound responsible for the root curvatures was the same as the one causing curvatures and malformations on the stems and petioles of bean plants. Whatman No. 3 paper was cut into strips (4 × 40 cm) and streaked 6.4 cm from the top of the paper with 0.1 ml of 10 × concentration of ether extract of *A. niger* culture filtrate. For purposes of comparison with a naturally occurring growth substance, similar papers were streaked with indoleacetic acid (IAA). A variety of solvents were used to develop the papers, by descending chromatography. When the solvent had moved 25 to 30 cm, the papers were dried and cut into strips 2 cm wide beginning 1 cm above the origin. These strips were eluted with 6 ml of 95 percent ethanol for 2 hours, the papers were removed, and the eluates were evaporated to dryness at 50°C in a forced air oven. The residue was taken up in 1 ml of water which contained four drops of Tween 80 per 100 ml and used to treat the growing points

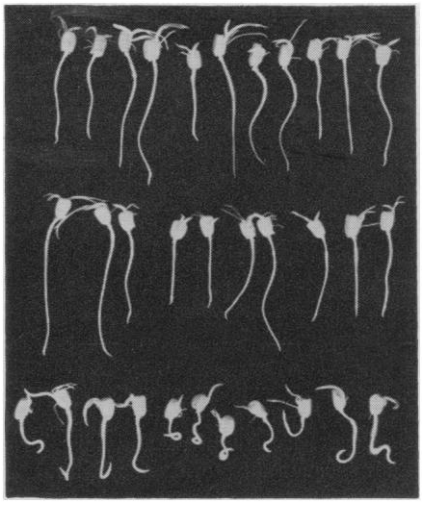

Fig. 1. Roots obtained from corn seeds germinated on filter paper moistened with water (top), ether extract of uninoculated culture medium (middle), and ether extract of *A. niger* culture filtrate (bottom).

Table 1. R_f values of IAA and of the compound produced by *A. niger* causing bean malformations and corn root curvatures, with various solvents.

Compound inducing bean malformations	Compound inducing corn root curvatures	IAA
	Water	
0.85	0.83	0.88
	Ethanol (70%)	
0.95	0.95	0.78
	Phenol (H_2O saturated)	
0.96	0.95	
	Isopropanol:NH_3:H_2O (10:1:1)	
0.93	0.93	0.41
	Pyridine:NH_3 (4:1)	
0.95	0.95	0.53
	Chloroform	
0.00	0.00	0.00
	Chloroform (H_2O saturated)	
0.46	0.46	0.17

of bean seedlings as described earlier (*1*). After treatment of the bean seedlings, the remainder of the eluate was diluted with an additional 9 ml of water and tested for corn root curvatures as described above. If the active compound was found to occur on each of two adjacent strips of the chromatogram, its movement was calculated midway between the two strips. Indoleacetic acid was located on the chromatograms with the ferric chloride–perchloric acid reagent (*2*). The R_f values obtained (Table 1) indicate that the compounds causing root curvatures and bean malformations are identical and have R_f values substantially different from those obtained for IAA (*3*).

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28 March 1958

Enzymatic Action of Rabbit Serum on Cortisone Acetate and Hydrocortisone Acetate

Enzymatic activity upon corticosteroids has been reported for tissues (*1*). The only known report, to date, on the enzymatic effect of serum per se is a paper by H. H. Wotiz *et al.* (*2*) on the metabolism of testosterone by human serum. We wish to report (*3*) the presence of an esterase in normal rabbit serum which is absent from normal human serum and which removes the acetate group from cortisone acetate and to a lesser degree from hydrocortisone acetate.

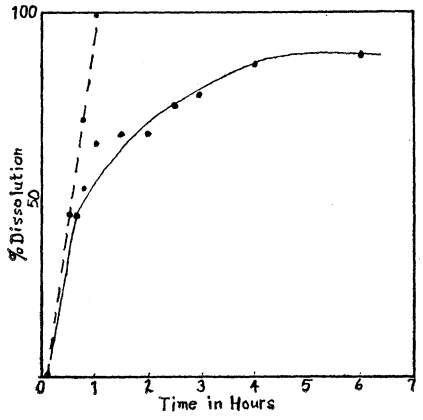

Fig. 1. Percentage dissolution of CA (dashed line) and HCA (solid line) in 10 percent normal rabbit serum in saline (NRS).

Following the initial studies in this laboratory on the effect of cortisone acetate upon the susceptibility of HeLa cells to poliovirus (*4*), the observation was made that in cultures maintained in Eagle's medium (*5*) with 10 percent human serum the cortisone acetate remained in particulate form, while in those cultures in which rabbit serum was substituted for the human serum the compound soon went into solution.

To investigate this phenomenon quantitatively, three sets of reaction tubes were initiated with 0.9 percent saline (SAL), 10 percent normal human serum in saline (NHS), or 10 percent normal rabbit serum in saline (NRS). To the tubes of each set were added either cortisone acetate (CA) (*6*), hydrocortisone acetate (HCA) (*6*), or hydrocortisone free alcohol (H-OH) (*6*), each resulting in a final concentration of 0.25 mg/ml. All tubes were incubated at 37°C. The hydrocortisone free alcohol was immediately soluble in all three reaction mixtures. The optical density of the tubes containing the steroid acetates was determined immediately and at intervals thereafter. Readings were made on the Bausch and Lomb Spectronic 20 with a wavelength setting of 560 mμ and were corrected with respect to homologous blanks. Figure 1 shows the percentage dissolution of the steroids with time.

Fresh rabbit serum was used in the experiments presented. Considerable activity could still be demonstrated, however, in serum stored at 4°C for 1 month. The heat lability of this enzyme is shown in the experiment summarized in Table 1. Fresh rabbit serum was heated at 56°C for 30 minutes. Comparable tubes were set up with NRS made with heated and unheated serum. Either HCA or CA was added at a final concentration of 0.25 mg/ml. Optical density values were followed with a Coleman, Jr. spectrophotometer with a wavelength setting of 560 mμ.

When a *p*H indicator such as phenol red was incorporated with the reaction mixture, it was seen that a *p*H drop from 7.4 to 6.8 occurred during the reaction period, indicating the formation of an acid. This did not occur in either the 0.9 percent saline or the 10 percent normal human saline reaction mixtures.

Samples of the NRS cortisone acetate reaction mixture were analyzed by the Merck Sharp & Dohme Research Laboratories. Their paper strip data showed that cortisone free alcohol was essentially the only form of cortisone present in the final reaction mixtures (*7*).

We conclude that normal rabbit serum contains an esterase capable of splitting CA into the free alcohol and acetic acid. Rabbit serum also has esterase activity toward hydrocortisone acetate. Human serum did not exhibit either esterase activity in the 24-hour test period.

Table 1. Optical density readings on Coleman, Jr. spectrophotometer. Normal rabbit serum unheated versus normal rabbit serum heated. All readings were corrected to a saline NRS blank of constant zero reading.

Time (hr)	CA, not heated	CA, heated	HCA, not heated	HCA, heated
0.00	0.32	0.31	0.20	0.20
0.25	0.28	0.31	0.18	0.20
0.50	0.22	0.31	0.17	0.20
0.75	0.12	0.31	0.097	0.20
1.00	0.00	0.31	0.097	0.20
2.00	0.00	0.31	0.097	0.20
3.00	0.00	0.31	0.097	0.20
24.0	0.00	0.31	0.097	0.20

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16 April 1958

Pericentral Cortical Projections to Motor and Sensory Nuclei

In an experimental neuroanatomical study in the cat (*1*) in which the Nauta-Gygax silver impregnation technique (*2*), was used, some of the corticobulbar fibers were found to be distributed (i) to the spinal trigeminal complex and the adjacent lateral tegmentum up to the level of the isthmus and (ii) to the region of the nuclei cuneatus and gracilis. No corticofugal fibers were distributed to the motor nuclei. Moreover, lesions within the limits of the cat's "motor cortex" (*3*) revealed that the projection to the spinal trigeminal complex and the adja-