antibody with antigen should be affected more adversely by increased negative charge than that with hapten, since the protein-protein electrostatic repulsion, in the case of the reaction with antigen, is superimposed on that involving the haptenic group. Additional, intermolecular repulsions may also be involved in the formation of aggregates of antibody and antigen.

Finally, it is of interest that the nonspecific electrostatic effect in the interaction of the antibody with hapten is small. With about 65 additional negative charges in the antibody molecule, the binding of *p*-iodobenzoate by antibody was affected only to a small degree (Table 1). In untreated antibody at *p*H 8, the net negative charge per molecule is about 15 or 16 units; thus the nonspecific electrostatic free energy of combination at *p*H 8 is probably negligible. This of course does not preclude the possibility of a very large interaction of opposite charges in the specific combining region.

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7. One milliliter of $10^{-6}M$ solution of *p*-iodobenzoate gave approximately 2000 count/min (about 15 times background). Counting efficiency was about 50 percent.
8. The small correction was made by use of the observation, made with a number of different preparations of this antibody (*4*), that the fraction of hapten bound, at hapten concentrations which are small compared to the antibody concentration, does not change appreciably with small changes in the free concentration of hapten.
9. For a given species of site (that is, group of sites having the same K) the concentration of hapten bound is directly proportional to the total concentration of those sites remaining, provided that K and the free hapten concentration are fixed. It follows that the concentration of hapten bound to a heterogeneous population of combining sites, under the same conditions, is proportional to the total concentration of sites. This assumes that acetic anhydride does not react selectively with sites on the basis of their combining constants.
10. Preliminary experiments indicate that the ability of the mildly acetylated antibody to bind the dye, *p*-(*p*-hydroxyphenylazo)-benzoate, is similarly unimpaired. Since this hapten is larger than *p*-iodobenzoate, this result supports the evidence against an attack on the specific combining region.
11. Dr. Schlamowitz of this laboratory, in studies on the phosphatase-rabbit antiphosphatase system, has found that acetylation of the antibody causes a delay in precipitation, but that the antibody still combines with antigen, as evidenced by coprecipitation of the complexes with horse antirabbit γ-globulin antibodies (unpublished results).
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Fluorescence Activation Spectra of a Diphosphopyridine Nucleotide Dependent Dehydrogenase

In a previous communication from this laboratory (*1*), the shift and augmentation of the fluorescence spectrum of reduced diphosphopyridine nucleotide (DPNH) (*2*) in the presence of beef heart muscle lactic dehydrogenase (LDH) was reported. It was further reported that an additional shift and increase of the fluorescence spectrum occurs when L-lactate is added to the LDH-DPNH complex, presumably to form an LDH-DPNH-L-lactate complex (*3*). For DPNH, when activated by light having a wavelength of 340 mμ, maximum fluorescence emission occurs at 465 to 470 mμ. For LDH-DPNH and LDH-DPNH-L-lactate complexes, maximum emission is observed at 445 to 450 mμ and 430 to 435 mμ, respectively. Similar shifts in the fluorescence spectrum have recently been reported for other dehydrogenase systems (*4*).

Since the initial observation that the alteration of the fluorescence spectrum of DPNH in the presence of horse liver alcohol dehydrogenase is accompanied by a shift to shorter wavelengths of the *absorption* maximum of DPNH (*5*) several attempts have been made to detect a similar alteration of the absorption spectrum of DPNH in the presence of LDH. However, the magnitude of the absorption change is so small that it could be detected only with the very sensitive spectrophotometer employed by Chance and Neilands (*6*).

Since it is well known that only absorbed light can give rise to fluorescence emission, it occurred to us that in the case of LDH and DPNH, only a small fraction of the absorbed light gave rise to the fluorescence spectrum. As a result, rather pronounced changes in the fluorescence spectrum are accompanied by minute changes in the absorption spectrum. In this case, examination of the activation spectrum should reveal those changes in the absorption spectrum which give rise to fluorescence emission (*7*). Figure 1 illustrates activation spectra of DPNH, LDH, LDH-DPNH complex, and LDH-DPNH-L-lactate complex as measured in the Aminco-Bowman recording spectrophotofluorometer (*8*) in 0.2 ionic strength phosphate buffer, *p*H 6.61, at 20°C. For DPNH, LDH-DPNH complex, and LDH-DPNH-L-lactate complex, the fluorescence monochromator was set at the wavelength of maximum emission. For LDH, the fluorescence monochromator was set at 465 mμ, the wavelength of maximum emission of DPNH. The addition of pyruvate to a final concentration of $1.3 \times 10^{-4}M$ to either the LDH-DPNH or LDH-DPNH-L-lactate systems results in a rapid change to the activation spectrum of LDH alone.

It is clear from Fig. 1 that the wavelength of maximum activation of the LDH-DPNH complex is about 5 mμ less than that of DPNH, and that a further shift of 5 mμ is observed when the LDH-DPNH-L-lactate complex is formed. A striking change in the activation spectrum of LDH is seen at 285 mμ when enzyme-coenzyme and enzyme-coenzyme-L-lactate complexes are formed. Ternary complexes are also formed when structural analogs of L-lactate, such as oxalic, tartaric, tartronic, α-hydroxybutyric, malic, and ascorbic acids, are

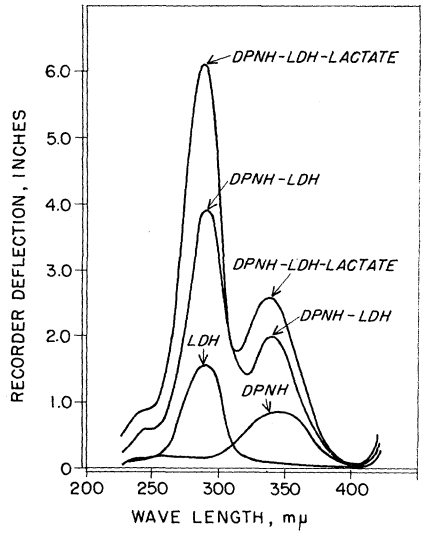

Fig. 1. Fluorescence activation spectra of lactic dehydrogenase and of lactic dehydrogenase complexes with DPNH and with DPNH and L-lactate. The intensity of fluorescence emission at a constant wavelength, as measured by recorder deflection, is plotted against the wavelength of the activating radiation. The curve labeled LDH was obtained with $9.60 \times 10^{-7}M$ LDH. The molecular weight of the enzyme was taken as 135,000 (*10*). The curve labeled DPNH was recorded at a DPNH concentration of $3.94 \times 10^{-6}M$. When DPNH and LDH were each present at the concentration used for the measurement of their separate spectra, the curve LDH-DPNH was obtained. The curve labeled LDH-DPNH-L-lactate was obtained when Na-L-lactate at a final concentration of $1.57 \times 10^{-2}M$ was added to LDH and DPNH present in the concentrations used for the other curves. The background fluorescence of phosphate buffer and L-lactate is negligible at the instrument settings employed.

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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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

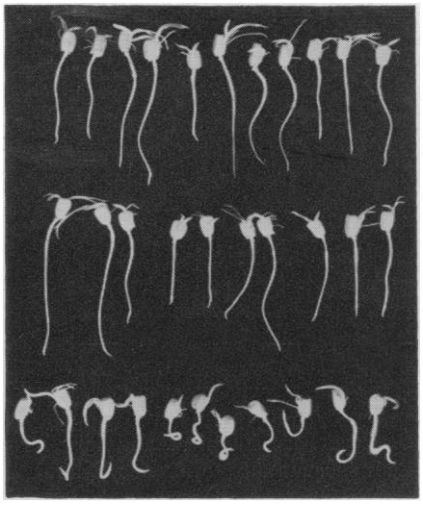

Fig. 1. Roots obtained from corn seeds germinated on filter paper moistened with water (top), ether extract of uninoculated culture medium (middle), and ether extract of *A. niger* culture filtrate (bottom).

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