morphological mutants. This very low rate of spontaneous mutation serves to emphasize the stability of the particular strain used.

The results of the treatments are summarized in Table 1. It is evident that the nitrogen mustard was relatively toxic to the spores and that this lethal action continued throughout the period of treatment. Furthermore, the data show that this nitrogen mustard was very effective in producing morphological mutants and that the percentage of mutants based on the number of survivors continued to increase throughout the duration of the treatment. The percentage of mutants based on the original number of spores plated out increased from 0.1 in the untreated control to 2.7 for the 4-minute treatment. This large increase indicates that the nitrogen mustard treatment actually produced these mutants and did not merely serve as an agent which preferentially allowed pre-existing mutants to survive. The subsequent decline in the total number of mutants also indicates this nonselective action.

The data obtained on irradiating a comparable spore suspension with a narrow band of ultraviolet radiation at 2,750 A. show that ultraviolet radiation of this wave length exerts a considerable toxic action and also induces mutations. However, in contrast to the nitrogen mustard treatment, the number of mutants based on the percentage of survivors reached a maximum and then declined. The maximum number of mutants produced was somewhat higher than that obtained by the nitrogen mustard treatment.

In so far as could be judged by visual examination of the colonies, there is no essential difference between the two treatments as to the types of mutants produced. Ultraviolet-induced mutants of NRRL-832 have been carried through repeated transfers without evidence of reversion to the original form. Representative mutants induced by nitrogen mustard were carried through a second transfer and some through successive transfers without observation of any change in morphological characters. Since the sexual stage of this organism is unknown, it is impossible to study the inheritance of these induced characters by the usual methods.

On the basis of the number of spores surviving treatment, it appears that the nitrogen mustard can be more effective than the ultraviolet radiation of the wave length employed in inducing mutants in this strain of *P. notatum*.

Studies dealing with the action of the nitrogen mustards on various races of *P. notatum* and *P. chrysogenum* are being continued in investigating the variability of these organisms in obtaining races showing biochemical differences such as changed nutritional requirements and capacity to produce penicillin.

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Separation and Immunologic Evaluation of Soluble Pertussal Antigens[1]

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Leslie and Gardner (*3*) demonstrated that *Haemophilus pertussis*, the causative agent of whooping cough, is monotypic and, under adverse cultural conditions, is progressively degraded from smooth Phase I to rough Phase IV strains, the latter phase being irreversible. The development of improved pertussal vaccines by Sauer (*9*) and Kendrick (*1*) followed these observations. Subsequent clinical data indicate that prophylactic efficacy is closely related to Phase I characters.

Until recently different pertussal vaccines could be evaluated only on the basis of clinical trial, since no satisfactory laboratory test existed. However, Kendrick (*2*) has now introduced an intracerebral challenge test in mice employing live Phase I organisms. It was shown that immunization intraperitoneally with a single dose of 100,000,000–500,000,000 Phase I organisms protects mice against 100–1,000 fatal intracerebral doses of live *H. pertussis*. If the assumption is made in this test that a correlation exists between mouse antigenicity and the prevention of whooping cough in human beings and, further, that the test might distinguish quantitatively the antigenic power of different pertussal vaccines or antigens, it should be possible to evaluate antigens, other than Phase I vaccine, for their prophylactic efficacy.

Accordingly, the present report concerns studies on the immunologic evaluation by the Kendrick test of soluble pertussal antigens (SA), supplied by Lederle Laboratories. The parent materials contain, among other things, detoxified thermolabile toxins (*7*), capsular or surface antigens (*5*), as well as detoxified somatic antigens (*5*). Improved methods for the preparation of parent SA will be published subsequently (*8*). The behavior of SA in methanol-water mixtures (*4, 6*) under controlled conditions of pH, ionic strength, and temperature is also presented.

The protective dose (PD_{50}) of the various antigens was determined by the Kendrick test. Groups of 10 mice each were injected intraperitoneally with 0.5 ml. of varying dilutions of SA or Phase I vaccine. After a rest period of 10 days, the mice were challenged intracerebrally with 100–1,000 fatal doses of Phase I *H. pertussis*. Control groups of 10 mice were employed to verify the adequacy of the challenge dose. The Standard Reed-Muench calculations were employed to determine the PD_{50} of the various antigens.

Nitrogen was determined in duplicate by the micro-Kjeldahl method of Pregl. Hydrogen-ion determinations were made on the glass electrode. Adjustment of the pH of SA for the fractionation with methanol was made with an acetate buffer (*4*) for hydrogen-ion concentrations less than pH 4.6, with acetic acid for pH 4.1, and with HCl for those greater than pH 4.1.

One volume of SA is chilled to 1° C. and adjusted to the desired pH with ice-cold buffer or acid and immediately transferred to −5° C. bath. To this mixture the calculated amount of methanol (measured at −5° C. and then chilled to −20° C.)

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is added slowly with stirring, care being taken to maintain the temperature at −5° C. The mixture is allowed to stand at −5° C.; the precipitate is removed in a refrigerated centrifuge at −5° C. and is freed of as much of the supernatant as possible by drainage. The precipitate is dissolved to 1/10 the original SA volume with ice-cold M/15 phosphate buffer of pH 7.4 and clarified by centrifugation at 4,000 r. p. m. for 15 minutes at 0° C. The purified SA samples were maintained at −30° C. until assayed.

Table 1 summarizes the results obtained with SA fractions separated between pH 2.0 and 7.0 in 40 per cent methanol at

TABLE 1

Conditions		Mg. N*/ ml.	PD_{50} (ml.)	PD_{50}'s/ mg. N	PD_{50} yield (%)†
pH	Methanol (%)				
2.0	40	0.31	0.6	5	3
3.1	40	0.47	0.06	35	27
4.1	40	0.45	0.017	131	94
4.6	40	0.36	0.025	111	64
5.1	40	0.30	0.042	79	38
6.0	40	0.21	0.041	116	39
7.0	40	0.17	0.10	59	16
4.1	25	0.35	0.16	18	10
4.1	10	0.27	0.11	34	15
Parent antigens		4.55	0.16	1.4	—

* Ten times the concentration of parent antigens.

† $\frac{\text{Total } PD_{50} \text{ precipitated}}{\text{Total } PD_{50} \text{ in parent antigens}} \times 100$.

−5° C. These data indicate that the substances responsible for the protection of mice against *H. pertussis* are quantitatively precipitated (within the limits of accuracy of the test) at pH 4.1 in 40 per cent methanol. Hydrogen-ion concentrations greater than pH 4.1 lead to an increased solubility of protective substances; those less than pH 4.1 also result in a progressive

TABLE 2

Antigen	PD_{50} (ml.)	No. of Phase I organisms/ PD_{50} (billions)
Purified SA, alum precipitated	0.004	—
Purified SA, alum precipitated + 5,000,000,000 Phase I Vaccine*	0.0026	0.013
Purified fluid SA	0.017	—
10,000,000,000/ml. Phase I Vaccine*	0.015	0.150

* Lederle # 2074-21.

loss of antigen into the supernatant fluid. On a nitrogen basis, the PD_{50} of the fraction separated at pH 4.1 has been purified about 95-fold over parent antigens. Further fractionation of this fraction has resulted in products of over 200-fold purity (*5*).

In other experiments it was noted that methanol concentrations under 40 per cent resulted in the incomplete precipitation of the antigenic factors. These experiments, as well as others to be reported later, suggest that two or more soluble antigens are responsible for the full protection of mice against *H. pertussis*. The above results have been duplicated with different lots of parent SA varying in PD_{50} from 0.09 to 0.29 ml.

Table 2 summarizes the results of a comparative study of the antigenic potencies of concentrated SA fractions separated at pH 4.1 in both the fluid and alum-precipitated state and of Phase I vaccine. The alum-precipitated sample is over 3 times more antigenic than the plain bacterial vaccine. The antigenicity of a mixture of alum-precipitated SA and 5,000,000,000 Phase I organisms was 5 times as great as that of the vaccine. Concentrated fluid SA compares favorably with a 10,000,000,000 Phase I vaccine.

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Some Effects of Ultraviolet Light on 2, 4-D and Related Compounds[1]

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Studies of the action of ultraviolet light on plant growth-regulating compounds have been limited. In 1938 Gilman, in studying the effect of ultraviolet light on unsaturated compounds (*3*), reported that ultraviolet light changed trans-cinnamic acid to a mixture of cis-cinnamic, truxillic and truxinic acids. A year later, Zimmerman and Hitchcock (*5*) showed that the relatively inactive trans-cinnamic acid could be changed to the active cis-cinnamic acid by ultraviolet light. Other workers have noted the effect of light on naturally occurring growth substances. It was shown by du Buy in 1933 (*2*) that white light (not specified) plus heat decreased the growth substance supply in the *Avena* coleoptile. The work of Boysen (*1*) suggested that auxin-a is inactivated, at least in part, by white light but that heteroauxin (indole-3-acetic acid) is not.

The objective of the study reported here was to determine the effect of ultraviolet light on 2,4-D, the sodium, ammonium, and triethanolamine salts, and the methyl, ethyl, and butyl esters. Also included were studies on 2-methyl-4-chlorophenoxyacetic acid. These compounds were selected because they were the active ingredients in most of the hormone-like herbicides available during 1945–46. The results of such a study should assist in the interpretation of comparative field tests when these compounds are used as weed killers.

The chemicals used in this study came from several sources. The 2,4-D (m.p., 139°C.) was prepared in the laboratory of the Chemistry Section, Colorado Agricultural Experiment

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