THE ROLE OF BACTERIAL ENDOTOXINS IN OCCUPATIONAL DISEASES CAUSED BY INHALING VEGETABLE DUSTS

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A large group of occupational diseases connected with the inhalation of various vegetable dusts, especially in the textile industry, have certain main symptoms in common such as fever, coughing, dyspnoea, and general malaise. In most cases the symptoms are more prominent on Mondays or on resuming work after one or more days of interruption. The symptomatology of these diseases and the Monday effect leads to the hypothesis that they are due to the inhalation of the endotoxins of gram-negative bacteria that contaminate the various vegetable materials, the Monday effect being connected with the phenomenon of tolerance to the endotoxins. Support for this view came from the demonstration of the constant presence of endotoxins in cotton dusts in textile mills and from the study of the effects of the inhalation of purified endotoxins in rabbits and man.

The pathological processes observed in workers exposed to the inhalation of different vegetable dusts are numerous and varied. Some of the diseases produced by these dusts have features in common such as attacks of fever, accompanied by respiratory disturbances, and the characteristic appearance or enhancement of symptoms on resuming work after one or more days of interruption, as on Mondays. These resemble some effects of the endotoxins of gram-negative bacteria, that are powerful pyrogens and are able to induce a local inflammatory response in the tissues; also the Monday effect might be compared to the ready loss of tolerance to endotoxins that follows discontinuation of exposure to these agents.

A reappraisal of the possible role of the endotoxins of gram-negative bacteria in the pathogenesis of occupational diseases caused by the inhalation of vegetable dusts, might be rewarding.

Presence of Endotoxins in Industrial Cotton Samples

One of the vegetable dusts most often recognized as responsible for occupational diseases is cotton dust. It was decided therefore to find out whether

endotoxins were present in cotton samples normally in use in the textile industry. The following experiments were carried out.

Pyrogenic Activity of Cotton Extracts.—Aqueous cotton extract, 1 ml., prepared at room temperature as indicated by Antweiler (1960) and by Bouhuys, Lindell, and Lundin (1960), when injected intravenously in a rabbit, is strongly pyrogenic. The fever curve has two peaks (Fig. 1) similar to that produced by intravenous injection of endotoxins. The pyrogenic effect was always present when many extracts were used, prepared from different samples of raw cotton and from cotton debris collected in the carding machines. It has been observed, however, that the relative potency of the extracts varied appreciably, the most active extract being that prepared from discarded cotton residues from the carding machines. The extraction of pyrogens from cotton with aqueous solutions is more efficient if the extraction is made at 100° C. instead of at room or ice-box temperature. Extraction with normal saline buffered at pH 7.4 at 100° C. for 90 minutes gives an extract about 10 times more pyrogenic than that prepared from the same amount of cotton extracted overnight at room temperature, with the same amount of fluid. This is interesting in that it is similar to the method used by Neter,

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Bertram, Zak, Murdock and Arbesman (1952) for extracting endotoxins from gram-negative bacteria.

The daily intravenous injection of cotton extract produces, in rabbits, a clear-cut tolerance to the pyrogenic effect (Fig. 1), similar to that occurring after daily intravenous injection of endotoxins. More important still is the observation that rabbits made tolerant to the pyrogenic action of cotton extracts are also tolerant to the intravenous injection of purified *S. abortus equi* endotoxin (Fig. 2), and that, on the other hand, animals made tolerant to the endotoxin are tolerant to cotton extracts (Fig. 3). This suggests that the pyrogenic activity of cotton extracts may be due to the presence of endotoxins.

Action of Cotton Extracts on Circulating Leucocytes.—The intravenous injection in the rabbit of 1 ml. of aqueous cotton extract also produces marked variations of circulating leucocytes. First there is a decrease in the number of leucocytes in the peripheral blood, followed by a marked leucocytosis (Fig. 4). Both variations are similar to those elicited by endotoxins (Delaunay, 1943).

Extraction from Cotton of Schwartzmann-active Material.—If the method of Westphal, Lüderitz, and Bister (1952), for the extraction of endotoxins from gram-negative bacteria with phenol solutions, is used for the extraction of cotton samples, variable amounts of water-soluble material are obtained. The amount of extracted material has been 3.3 mg. from 1 g. of raw ordinary cotton (U.S.A. "good middling") and 9.9 mg. from 1 g. of waste cotton from the carding machines. Chemical analysis showed that in both cases the extracted material

FIG. 1.—Fever patterns induced by 1 ml. intravenous cotton extract in three untreated rabbits (—) and in two rabbits (---) after seven days of daily treatment with the same extract.

FIG. 2.—Fever patterns induced by 0.05 γ intravenous *S. abortus equi* endotoxin in four untreated rabbits (—) and by 5 γ of the same endotoxin in two rabbits (---) injected daily (seven days) with 1 ml. cotton extract.

FIG. 3.—Fever patterns induced by 1 ml. intravenous cotton extract in three untreated rabbits (—) and in five rabbits (---) made tolerant to 20 γ of *S. abortus equi* endotoxin.

FIG. 4.—Mean value of percentage variations in white blood cell count in three rabbits after intravenous injection of 1 ml. cotton extract.

had a low nitrogen content (1.2% in the first and 3% in the second case) and was comparatively rich in hexoses (17% in both cases).

This material was pyrogenic. The minimal dose capable of increasing the rectal temperature of rabbits by 0.6°C. was 0.2 µg., and it was Schwartzmann active. In fact the intracutaneous injection of 30 µg. of the material extracted from cotton was sufficient to prepare the rabbit for a local Schwartzmann reaction subsequently provoked by the intravenous injection of 50 µg. of purified *E. coli* endotoxin.

Our experiments show consistently the presence, in industrial cotton, of material with biological activities similar to those of the endotoxins of gram-negative bacteria. A rough estimate, based on the pyrogenic activity, indicates that 1 ml. of aqueous cotton extract prepared as indicated by Antweiler (1960) may contain between 10 and 30 µg. of endotoxin-like material (*i.e.* 0.15–0.50 mg. extracted from 1 g. of cotton). A more precise assessment is derived from the extraction of cotton by the method of Westphal, Lüderitz, Eichenberger, and Keiderling (1952). As stated above, this method indicates that 1 g. of waste cotton from the card-rooms may contain about 10 mg. of endotoxin-like material.

We have examined the following samples for the presence of endotoxins: three samples of industrial raw cotton (one "good middling" and one "good ordinary" from the U.S.A. and one of Egyptian cotton), one sample of raw beaten flax, and one of hemp. All these materials contained different amounts of endotoxins, ranging from 3 mg. to 11 mg. per gramme.

It will obviously be useful to determine the endotoxin content of many more samples of cotton of differing grade and origin, and also of other raw natural textile fibres. It is already apparent that the presence of bacterial endotoxins is not limited to some samples of very low-grade cotton, where they have already been shown to be present by Neal, Schneider, and Caminita (1942).

The origin of the endotoxins found in cotton and other natural textiles can probably be ascribed to the various gram-negative saprophytic bacteria that have developed on the cellulose-rich textiles during the whole period extending from the crop in the fields to processing in the factories. It is reasonable to assume that only a part of these bacteria are still viable in the raw cotton ready for industrial processing. Furness and Maitland (1952) found between 12 and 252 millions of viable gram-negative bacteria (mainly *Aerobacter*) per gramme of different samples of raw cotton. The number of viable gram-negative bacteria found in

a sample of cotton or other textiles is not a reliable measure of its endotoxin content, for one must take into account the endotoxins connected with the residues of any dead bacteria that may have remained adhering to the cotton fibres.

One would expect there to be variations in the amount and origin (from different species of gram-negative bacteria) of the endotoxins present in different samples of cotton or other textiles, depending on the varying conditions to which the individual sample is subjected, while still in the crop, during storage and processing. It may be that larger amounts of endotoxins will be found associated with cottons of lower commercial grade that are presumably exposed to more extensive bacterial contamination than the higher grades.

The Relationship between some Experimental Effects of Cotton Extracts and those of Endotoxins

The biological effects of cotton extracts have been studied fairly extensively in experimental animals and in man. Of special interest is the recent work of Bouhuys *et al.* (1960) and of Antweiler (1960), showing the presence in cotton extracts of a substance or substances which are histamine liberators. The demonstration of endotoxins in cotton extracts raises the question as to whether some of the effects observed with cotton extracts, might be due to the endotoxins. Several instances in which this possibility may be considered are listed in Table 1, and will be briefly discussed.

Late Skin Reaction in 90% or More of Normal Subjects.—Cayton, Furness, and Maitland (1952) observed that several cotton extracts, when injected intracutaneously in man, induced a late reaction in almost all subjects. This effect was ascribed by these authors to the presence of a toxic substance, which may have been the endotoxins; it is well known that the intracutaneous injection of very minute amounts of endotoxins induces, after eight to 15 hours, a localized reddening and a slight infiltration in almost all subjects (Thomas, 1954).

Infiltration of Skin with Polymorphonuclear Leucocytes.—Prausnitz (1936) observed, in a self-experiment, that the intradermal injection of several cotton extracts produced a very marked and mainly perivascular infiltration of the dermis by polymorphonuclear leucocytes. A similar reaction was observed in the rabbit. Now this local leucocytic infiltration is one of the characteristic responses to the intradermal injection of endotoxins, and indeed is the morphological basis for the Sanarelli-Schwartzmann phenomenon (Thomas, 1954).

TABLE 1
COMPARISON OF SOME PHARMACOLOGICAL EFFECTS OF COTTON EXTRACTS AND OF ENDOTOXINS

Observed Phenomenon	Cotton Extracts	Endotoxins
Late skin reaction in > 90% normal people	Prausnitz (1936) Cayton <i>et al.</i> (1952)	See Thomas (1954)
Infiltration of skin with leucocytes	Prausnitz (1936)	See Thomas (1954)
Anaphylactoid shock at first intravascular injection in the guinea-pig	Prausnitz (1936)	
Fall in peripheral blood pressure and respiratory disturbances in the cat, antagonized by anti-histamine drugs	Bouhuys <i>et al.</i> (1960)	Kuida <i>et al.</i> (1958)
Liberation of histamine from the peritoneal cavity of the rat	Antweiler (1960)	Gilbert (1959)
Increase in plasma histamine	Antweiler (1960)	Our observation
Liberation of histamine from heparinized rabbit blood	Antweiler (1960)	Weil and Spink (1957)
Fall of circulating platelets	Antweiler (1960)	Our observation
Activation of anaphylatoxin in heparinized rat plasma	Antweiler (1960)	Davis <i>et al.</i> (1960)
Oedema of the foot-pad in the rat	Our observation Antweiler (1960)	Greisman (1960) Our observation

Primary Toxic Action after Intravascular Injection.

—Prausnitz (1936) obtained a toxic material from raw cotton, by aqueous extraction followed by precipitation with ethanol or ammonium sulphate, that proved to be lethal to some guinea-pigs when administered by intravascular injection. Definite anaphylactoid symptoms were observed in the animals. This active material was named “toxic protein” by Prausnitz, although it did not behave chemically as a pure protein. It appears probable that Prausnitz’s “toxic protein” contained a fair amount of crude endotoxic protein-lipid-polysaccharide complexes, and that these were responsible for the toxic activity of the preparation.

Fall of Peripheral Blood Pressure and Respiratory Disturbances in the Cat.—These reactions were observed by Bouhuys *et al.* (1960) and by Antweiler (1960) in the anaesthetized cat shortly after an intravenous injection of aqueous cotton extracts. Many workers have observed similar effects in the cat after intravenous injection of endotoxins. Indeed, if the dose of endotoxins is not too high, the action on blood pressure may be inhibited by antihistaminic drugs (Gilbert, 1959), as is the case with cotton extracts.

Liberation of Histamine from the Peritoneal Cavity of the Rat.—This effect was observed by Antweiler (1960) after the intraperitoneal injection of 15 ml. of cotton extract. We have seen a similar liberation of histamine from the peritoneal cavity of rats after 10 ml. Ringer solution containing 50 µg./ml. of endotoxin had been injected intraperitoneally. This liberation occurred with endotoxins from *E. coli*, *S. abortus equi*, and *S. typhi*. This amount of endotoxin is approximately the same as that probably present in 15 ml. of cotton extract.

Increase of Histamine-like Substances in Plasma.—An increase in histamine or histamine-like substances in the plasma of dogs treated with comparatively

large amounts of endotoxins has been observed by Weil and Spink (1957). A similar increase of histamine was observed by Antweiler (1960) in the blood of cats treated with cotton extracts.

Liberation of Histamine or Histamine-like Substances from Heparinized Rabbit Blood.—We incubated 2.5 ml. of heparinized (100 µg. heparin/ml.) rabbit blood for 30 minutes at 37°C., with 1 ml. of the following solutions, siliconed glassware being used throughout:

- normal saline;
- extract from card-room waste cotton prepared in normal saline according to the procedure of Antweiler (1960) and Bouhuys *et al.* (1960);
- the same extract filtered through LSG₆₀ membrane filters (Membranfilter, Göttingen);
- normal saline containing 25 µg./ml. or 50 µg./ml. of *E. coli* endotoxin;
- normal saline containing 25 µg./ml. of *S. abortus equi* endotoxin.

After incubation the rabbit blood was centrifuged and 0.25 ml. of the supernatant fluid was transferred to a vessel of 15 ml. capacity containing a segment of terminal guinea-pig ileum. The ileum was immersed in oxygenated Ringer solution and kept at 37°C., a small amount of atropine (1 mg./litre) having been added. The contraction of the guinea-pig ileum was recorded on a revolving drum. The results of a typical experiment are shown in Fig. 5. They confirm the observation of Antweiler (1960) concerning the ability of crude cotton extracts to liberate histamine or histamine-like substances from whole heparinized rabbit blood.

Fig. 5 shows, moreover, that the active substance or substances in cotton extracts are mainly withheld by Membranfilters LSG₆₀ which do not allow the passage of molecules of molecular weight appreciably greater than 10,000.

They also show that histamine or histamine-like substances (contraction of the ileum inhibited by 1 µg./ml. of pyribenzamine) are liberated by incubation of heparinized rabbit blood with *E. coli*

FIG. 5.—Action on the isolated guinea-pig ileum of supernatant from heparinized rabbit blood incubated (2.5 ml. blood + 1 ml. indicated solution/litre) with:

- (1) Cotton extract (crude) in saline;
- (2) Cotton extract filtered through Membranfilter LSG₆₀;
- (3) *E. coli* endotoxin in saline, 25 γ /ml.;
- (4) *E. coli* endotoxin in saline 50 γ /ml.;
- (7) Normal saline;
- H = 0.5 γ histamine.

endotoxin; surprisingly enough no effect was observed after incubation with *S. abortus equi* endotoxin.

Fall of Circulating Platelets.—Antweiler (1960) showed that the intravenous injection of cotton extracts produced a marked fall of circulating platelets in the rabbit. This might be due to the endotoxins present in cotton extracts in view of the dramatic reduction of blood platelets after the intravenous injection of purified endotoxins (Davis, Meeker, and McQuarrie, 1960).

Activation of Anaphylatoxin in Heparinized Rat Plasma.—Greisman (1960) has shown that activation of anaphylatoxin can be achieved by incubating fresh heparinized rat plasma with small amounts of *E. coli* endotoxin. To find out whether a similar effect might be obtained with cotton extracts, we incubated different crude cotton extracts in normal saline for 30 minutes at 37° C., prepared as indicated by Antweiler (1960), with equal amounts of fresh heparinized (100 μ g./ml.) rat plasma. After incubation the presence of activated anaphylatoxin in the rat plasma was investigated by its action on freshly prepared guinea-pig ileum as indicated by Rothschild and Rocha e Silva (1954).

The results of a typical experiment show that rat plasma anaphylatoxin can be activated by a crude cotton extract (Fig. 6). As was to be expected no anaphylatoxin activation was obtained with a solution of compound 48/80. The anaphylatoxin

FIG. 6.—Action on the isolated guinea-pig ileum of heparinized rat plasma incubated with equal amounts of:

- (1) Agar (Difco) 0.1 mg./ml. normal saline;
- (2) Cotton extract (crude) in normal saline;
- (8) Normal saline;
- (9) 25 γ 48/80 in normal saline;
- H = 1 γ histamine.

experiment thus demonstrates one instance in which the histamine liberating activity of cotton extracts is comparable with that of endotoxins, and different from that of low molecular weight histamine liberators such as compound 48/80.

That the anaphylatoxin activating substance (or substances) of cotton extracts are molecules of considerable size is shown by the complete inactivity of filtrates passed through LSG₆₀ Membranfilters.

Oedema of the Foot-pad in the Rat.—A further similarity between the action of cotton extracts and that of endotoxins is shown by the ability of both to induce the rapid production of oedema in the foot-pad of the rat. Antweiler (1960) demonstrated this for cotton extracts, and we have shown that the injection of 50 μ g. *E. coli* endotoxin dissolved in 0.1 ml. saline and injected into the dorsal side of the foot-pad of the rat induces, after 20 to 30 minutes, a marked oedematous swelling. If the animal had previously received an intracardiac injection of 1 ml. of a 1% Evans blue solution in saline, then the oedematous zone stained blue, due to the free passage of the Evans blue molecules through capillaries whose permeability was abnormally high.

The above facts and considerations suggest that in evaluating the pharmacological effects of cotton extracts their endotoxin content must be considered. They certainly do not prove that all the biological activity of cotton extracts is due to

endotoxins. Indeed, there is some evidence against this hypothesis. For instance, the ability of cotton extracts to liberate histamine from whole heparinized rabbit blood seems to be appreciably greater than that produced by their endotoxin content. Furthermore this ability is not entirely abolished (Fig. 5) by the filtration of cotton extracts through LSG₆₀ Membranfilters, which should retain all the endotoxins. It is quite reasonable to assume that crude cotton extracts might contain other pharmacologically active substances (including histamine liberators) which are different from the endotoxins. The nature of these hypothetical substances is not known but it will not be surprising if crude cotton extracts are shown to contain various polysaccharides generally similar, at least in their ability to liberate histamine, to the glucose polymer dextran. It is of interest that even dextrans of comparatively low molecular weight (down to 14,000) retain their ability to liberate histamine from heparinized rabbit blood (Haining, 1955).

Effects of Inhalation of purified Endotoxins or Cotton Extracts—Experiments on Rabbits

A crucial point in our work is the assumption that endotoxins are active if absorbed by inhalation and that their action is similar to that of inhaled cotton dusts or extracts. To investigate this point we have given healthy hybrid rabbits (1.5 to 2 kg. body weight) of both sexes aerosol inhalations of purified endotoxins or cotton extracts.

Inhalation of Endotoxins.—Many experiments were performed on a total of 43 animals, with different doses and different kinds of endotoxins administered at different intervals, but they need not be reported here in detail. It is sufficient to say that in most experiments the animals were made to inhale an aerosol of a solution containing 15 µg./ml. *S. abortus equi* endotoxin in saline for 15 minutes. During these 15 minutes 4 ml. of the solution were aerosolized, an unknown amount of solution being actually absorbed by the animals.

The main effect of this inhalation was the appearance of fever and dyspnoea in a variable percentage of the animals (Fig. 7). The fever response was usually equivalent to an increase in rectal temperature of 1 to 1.5° C. The dyspnoea was

often clearly evident and was recorded with spirographic tracings (Fig. 8a and b). Both fever and dyspnoea were usually observed from 30 to 50 minutes after the end of the inhalation.

Both fever and dyspnoea appeared occasionally after the first inhalation of endotoxins, but became more frequent after the second, third, and so on. Fever or dyspnoea only appeared if the new inhalation was performed after an interval of some days from the preceding one. If the inhalations took place on two consecutive days, then the second one of the two was consistently without effect, regardless of the previous exposure of the individual rabbit and of its tendency to respond with fever and dyspnoea. This is presumably due to the well-known phenomenon of tolerance to the endotoxins, and is reminiscent of the Monday effect which is characteristic of many diseases connected with the inhalation of textile or other vegetable dusts.

Inhalation of Cotton Extracts.—The animals inhaled a crude cotton extract in saline prepared as indicated by Antweiler (1960). Neither fever nor dyspnoea was ever observed after this treatment, and this is in accord with the previous observations of Neal *et al.* (1942) who did not observe in different animals (including rabbits) symptoms that the inhalation of cotton dust or extracts readily produced in man and baboons. If, however, rabbits previously "sensitized" by inhalation of endotoxins were exposed to the inhalation of cotton

FIG. 7.—Percentage of rabbits showing indicated alterations after inhalation of endotoxins or cotton extracts.

FIG. 8a.—Spirogram of rabbit No. 56
1 = untreated; 2 = 30 min. after second inhalation of endotoxin aerosol (50 γ /ml.); 3 = 60 min. after.

FIG. 8b.—Spirogram of rabbit No. 23
1 = untreated; 2 = 30 min. after the sixth inhalation of endotoxin aerosol (35 γ /ml.); 3 = 60 min. after.

extract after a suitable interval, a substantial number of them showed fever (Fig. 7). The effect of repeated and suitably spaced inhalations of cotton extracts alone has not yet been investigated.

Inhalation of Endotoxins; Experiments on Man

Neal *et al.* (1942) gave a brief description of the effects observed in a subject who was exposed to the inhalation of an aerosol made from the sterile filtrate of a culture of *Aerobacter cloacae*, a gram-negative bacterium known to produce an endotoxin. About 45 minutes after the end of the inhalation they observed the onset of fever, cough, dyspnoea, diffuse aches and nausea. We inhaled aerosols of purified endotoxins. In the first experiment three of us inhaled about 3 ml. of saline containing 5.10 and 20 μ g./ml. of *E. coli* endotoxins respectively. The immediate effects were dry coughing and shortness of breath, accompanied by a slight reduction of the one second timed vital capacity, measured with a spirometer by the method of Tiffeneau and Drutel (1952). After four days two of the subjects again inhaled 3 ml. of an aerosol of a solution containing 20 μ g./ml. of *S. abortus equi* endotoxin; the symptoms were the same with the addition of slight fever (to 37.5° C.) and a definite feeling of malaise. The third subject, who had inhaled only the 10 μ g./ml. dose of *E. coli* endotoxin, had developed (probably because of a condition of hypersensitivity), a severe reaction with a diffuse skin rash and high fever, and this

prompted the discontinuation of the experiments. An investigation of the possible effects of inhalation of endotoxins was then undertaken amongst the workers in a pharmacological firm in Milan where typhoid vaccines were manufactured. We found that while the centrifuging killed *S. typhi* (an operation performed about once every three months involving the centrifuging of considerable amounts of bacterial suspensions in a Sharples centrifuge) the conditions were sometimes such that an appreciable quantity of bacteria was dispersed in the air and subsequently inhaled by the workers. Amongst these the onset of fever (to 39.5° C.) was then regularly observed, and one of the workers had also shown a diffuse scarlatiform skin rash on one occasion. But the most interesting fact was that all the subjects with spontaneous bronchial affections, and especially those with a history of chronic bronchitis, had to be excluded from the rooms where the typhoid vaccine was manufactured because they often had asthmatic attacks, especially when centrifuging bacterial suspensions.

Occupational Diseases in the Pathogenesis of which Endotoxins appear to Play a Part

Inhaled endotoxins may have a role in the pathogenesis of occupational diseases connected with the inhalation of vegetable dusts. These diseases often appear in small epidemics and display a somewhat uniform symptomatology, the main symptoms being attacks of fever, coughing,

dyspnoea, headache, nausea, diffuse aches, and malaise. These symptoms are strikingly similar to the effects of endotoxins. Usually, there is no alteration in the chest radiograph and recovery is complete within one or two days. In some instances, however, such as "farmer's lung" and bagassosis a mottling often appears in the chest radiograph and recovery may take weeks to complete. This may be due to the inhalation of larger amounts of endotoxins. When exposure to the inhalation of vegetable dusts is repeated daily, as with many workers in the textile industry, the symptoms usually appear, or are more severe, upon resuming work after one or more days of absence, such as on Mondays.

Table 2 gives a synopsis of the occupational diseases that we believe may be connected with the inhalation of endotoxins.

The main symptoms of these diseases bear a superficial resemblance to those of the allergic reactions of the asthma-hay fever group with which they have often been included. We feel, however, that this inclusion is not justified. In fact, a specific hypersensitivity for a given antigen has never been conclusively shown in any of the diseases listed in Table 2, neither with skin tests nor with other immunological methods. Moreover these diseases generally involve numerous subjects who are engaged in the same work and seem to be connected with the inhalation of considerable amounts of the noxious substances, while the allergic conditions of the asthma-hay fever type are associated with the peculiar reactivity of some individuals and are elicited by contact with minimal amounts of the

specific allergen. Also, as has been pointed out by Schilling (1956), the tolerance phenomenon that gives the characteristic Monday effect is not observed in cases of allergic asthma, even if this has an occupational origin.

It is more reasonable to assume that the vegetable dusts listed in Table 2 all contain, or are contaminated with, a toxic substance or substances whose biological effects are similar to the symptoms of an allergic reaction; thus we agree with Bouhuys *et al.* (1960) and with Antweiler (1960) whose work has shown that cotton dusts in textile mills contain a toxic substance or substances which are histamine liberators. We believe that at least part of the histamine-liberating activity of cotton extracts is due to their endotoxin content.

Thus the pharmacological activity of cotton extracts is mainly due to contamination with bacterial substances and not to a product of cotton itself. Indeed the fact that active substances capable of inducing similar pathological reactions in man are apparently present in various vegetable materials deriving from taxonomically different plants such as cotton, sugar cane, grain, malt, flax, hemp, and others, is by itself suggestive that the origin of these active substances is probably due to a contaminant. Also occupational diseases very similar to the so-called "mill fever" or "Monday fever" of cotton workers, have been observed amongst operatives who had never been exposed to cotton or other textile dusts, but who were certainly exposed to the inhalation of endotoxins. In fact it appears that cases of Monday fever are not rare amongst the personnel of

TABLE 2
SYNOPSIS OF OCCUPATIONAL DISEASES POSSIBLY DUE TO THE INHALATION OF ENDOTOXINS

No.	Disease	Origin	Affected Workers	Monday Effect	Described by
1	Mill fever (Monday fever)	Cotton	New card-room or other cotton workers—endemic	+	Many authors since 1784
1 (a)	Byssinosis	Cotton	Old card-room workers	+	Many authors
2	Weaver's cough	Cotton	Weavers—epidemic		Collis (1915) Middleton (1926) Vigliani <i>et al.</i> (1954) Neal <i>et al.</i> (1942) (endotoxin role ascertained)
3	Mattress maker's fever	Low-grade cotton	Mattress makers—epidemic, severe		
4	Hemp fever	Hemp	Hemp beaters—endemic	+	
5	Hackling fever	Flax		+	
6	Grain fever	Grain	Silo workers		
7	Malt fever	Malt			
8	Bagassosis	Bagasse (sugar cane)	Bagasse workers—endemic, severe		Role of endotoxins ascertained by Schneider <i>et al.</i> (1948)
9	Farmer's lung or Thresher's lung or Harvester's lung	Hay	Farmers—endemic, severe		Many authors; see Frank (1958)
10	Bible printer's fever	Humidifier + cellulose dust	Printers—epidemic		Contamination with gram-negative bacteria and role of endotoxins ascertained by Tejning and Grubb (1959)
11	Befeuchterungsanlage Fieber	Humidifier + sawdust	Wood model makers—epidemic	+	Pestalozzi (1959)

laboratories continuously engaged in the extraction and purification of endotoxins (Westphal, 1959).

Tejning and Grubb (1959) observed a small epidemic of "Monday fever" amongst printers working in a room equipped with an humidifier in which the water had become contaminated with gram-negative bacteria and contained endotoxins. An almost identical observation had previously been made by Pestalozzi (1959) in a Swiss factory manufacturing wood models. Pestalozzi ascribed the cause of attacks of fever and coughing occurring on Mondays to the contamination of the water of an humidifier ("befeuchters Fieber"), but he did not perform a complete bacteriological analysis of the contaminated water and thought that moulds were the cause of the disease, through an allergic mechanism. That bagassosis and a peculiar epidemic of fever and coughing observed amongst workers handling very low grade cotton for making mattresses were indeed due to endotoxins was demonstrated by Schneider, Reinhart and Caminita (1948) and by Neal *et al.* (1942). Our demonstration of the constant presence of endotoxins in cotton supports the thesis that the diseases of cotton operatives showing fever and cough (mill fever, weaver's cough) are also due to the endotoxins. In weaver's cough it is necessary to consider the presence of endotoxins not only in the cotton itself, but also in the size in which the threads are immersed before weaving. The process of sizing, performed in aqueous solutions at high temperature, might dissolve a good deal of endotoxins from the bacterial bodies, a process which is similar to the extraction of endotoxins with hot aqueous buffers (Neter *et al.*, 1952). Thus the sized threads might even be sterile, in so far as viable bacteria are concerned, but still be rich in the endotoxins that have dried on them. This agrees with the facts observed by one of us (Vigliani, Parmeggiani, and Sassi, 1954) in an epidemic of "weaver's cough".

There is no doubt that in weaver's cough, as in other instances, contamination of cotton or other materials is not restricted to gram-negative bacteria; yet we claim that the products of the gram-negative bacteria play a major role in the pathogenesis of the diseases because the observed symptoms and the Monday effect match well with the known effects of endotoxins.

Moulds are among the main contaminants of many cellulose-rich materials, particularly if moist, and to them or to their products some authors have ascribed the aetiology of certain occupational diseases connected with the inhalation of vegetable dusts (for example, farmer's lung, weaver's cough, and "befeuchters Fieber"). However, no proof

of this assumption has been given, and we wish to point out that moist cellulose-rich materials not only favour the development of moulds, but also of gram-negative bacteria of the aerogenes-capsulatus group that are often able to ferment the polysaccharides.

The Problem of Byssinosis.—The differences that exist between byssinosis and the diseases of the "mill fever" group, have been pointed out by Schilling (1956). Two of these differences, namely the absence of fever in byssinosis and the fact that years of exposure to cotton dust appear necessary for byssinosis to occur, are not easily reconciled with the hypothesis that endotoxins play a major role in the pathogenesis of byssinosis. On the other hand the patients affected by byssinosis in the earlier stages of the disease show a typical tolerance to the effects of the materials inhaled in the cotton factories, because they are worse on Monday as are those affected by mill fever or similar diseases. It may be, as already indicated by Neal *et al.* (1942), that byssinosis is due to protracted inhalation of small amounts of endotoxins.

This might result in a condition of immunological hypersensitivity, retaining some of the aspects of the reaction to endotoxins, such as tolerance.

More experimental work, coupled with observations on man, will be necessary in order to test this possibility.

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REFERENCES

- Antweiler, H. (1960). *Arch. Gewerbepath. Gewerbehyg.*, **17**, 574.
 Bouhuys, A., Lindell, S. E., and Lundin, G. (1960). *Brit. med. J.*, **1**, 324.
 Cayton, H. R., Furness, G., and Maitland, H. B. (1952). *Brit. J. Industr. Med.*, **9**, 186.
 Collis, E. L. (1915). *Proc. roy. Soc. Med.*, **8**, Sect. Epidemiol. and State Med., p. 108.
 Davis, R. B., Meeker, W. R., and McQuarrie, D. G. (1960). *Circulat. Res.*, **8**, 234.
 Delaunay, A. (1943). *C. R. Soc. Biol. (Paris)*, **137**, 589.
 Frank, R. C. (1958). *Amer. J. Roentgenol.*, **79**, 189.
 Furness, G., and Maitland, H. B. (1952). *Brit. J. industr. Med.*, **9**, 138.
 Gilbert, R. P. (1959). *Proc. Soc. exp. Biol. (N. Y.)*, **100**, 346.
 Greisman, S. E. (1960). *Ibid.*, **103**, 628.
 Haining, C. G. (1955). *Brit. J. Pharmacol.*, **10**, 87.
 Kuida, H., Hinshaw, L. B., Gilbert, R. P., and Visscher, M. B. (1958). *Amer. J. Physiol.*, **192**, 335.
 Middleton, E. L. (1926). *J. industr. Hyg.*, **8**, 436.
 Neal, P. A., Schneider, R., and Caminita, B. H. (1942). *J. Amer. med. Ass.*, **119**, 1074.
 Neter, E., Bertram, L. F., Zak, D. A., Murdock, M. R., and Arbesman, C. E. (1952). *J. exp. Med.*, **96**, 1.

- Pestalozzi, C. (1959). *Schweiz. med. Wschr.*, **89**, 710.
 Prausnitz, C. (1936). *Spec. Rep. Ser. med. Res. Coun. (Lond.)*, No. 212.
 Rothschild, A. M., and Rocha e Silva, M. (1954). *Brit. J. exp. Path.*, **35**, 507.
 Schilling, R. S. F. (1956). *Lancet*, **2**, 261.
 Schneider, R., Reinhart, W. H., and Caminita, B. H. (1948). *J. industr. Hyg.*, **30**, 238.
 Tejning, S., and Grubb, R. (1959). Private communication.
 Thomas, L. (1954). *Ann. Rev. Physiol.*, **16**, 467.
 Tiffeneau, R., and Drutel, P. (1952). *Presse méd.*, **60**, 640.
 Vigliani, E. C., Parmeggiani, L., and Sassi, C. (1954). *Med. d. Lavoro*, **45**, 349.
 Weil, M. H., and Spink, W. W. (1957). *J. Lab. clin. Med.*, **50**, 501.
 Westphal, O. (1959). Private communication.
 —, Lüderitz, O., and Bister, F. (1952). *Z. Naturf.*, **7b**, 148.
 —, Eichenberger, E., and Keiderling, W. (1952). *Ibid.*, **7b**, 536.

Addendum

It is interesting to point out that Paine (1946) observed, in four subjects exposed to the experimental inhalation of "*Serratia marcescens*", the appearance of an acute illness with coughing and

fever. He ascribed these symptoms to the endotoxin of "*Serratia marcescens*".

REFERENCE

- Paine, T. F. (1946). *J. infect. Dis.*, **79**, 226.