most studies designed to determine the etiologic agent in byssinosis have relied on in vitro test for normal volunteers to assess the effects of components isolated from cotton plants, cotton factory dust, or their associated organisms. In one of the few studies directly on byssinotic subjects, symptoms were reproduced on inhalation challenge with a condensed polyphenol isolated from bracts of cotton plants. The results were highly significant, with only byssinotic workers responding; however, when we repeated the investigation with 29 subjects, no individual responded to challenge with symptoms such as those experienced returning to work at the cotton mill after a weekend break. Tests of pulmonary function could not detect differences between polyphenol and saline challenges. Compared with groups of workers not exposed to cotton dust and matched for age and smoking habits, significant differences in pulmonary function were observed, confirming the detrimental effects of long-term exposure to cotton dust, particularly in combination with cigarette smoke.

The condensed polyphenol, which is polymerized leucocyanadin\(^1\)(5, 7, 3', 4'-tetrahydroxyflavan-3, 4-diol) and related to tannin, has produced the symptoms of byssinosis in five of six byssinotic subjects upon inhalation challenge but no symptoms in 11 nonbyssinotic cotton workers or unexposed subjects.\(^6\) Endotoxin may cause a reduction in FEV\(_1\), in some subjects,\(^10\) but this is just one example of the plethora of biologic activities it may exhibit.\(^11\)

Since inhalation challenge is recognized as the most direct diagnostic avenue for organic dust diseases, and due to the highly significant results previously obtained with condensed polyphenol,\(^9\) it was considered important to repeat the study with a larger byssinotic population.

**Materials and Methods**

**Byssinotic Subjects**

Subjects were obtained via the medical officer (H.B.) for the Iraqi State Establishment for Textiles at Baghdad from the 6,000-employee plant producing only cotton products from cotton bolls grown mainly in the Mosul district of Iraq. Twenty-nine male workers were asked to participate, 11 smokers (more than ten cigarettes per day), 11 nonsmokers, and seven ex-smokers. All had symptoms of grade-1 or grade-2 byssinosis\(^12\) on the basis of the tightness in the chest and shortness of breath occurring on the first day back to work (Saturday) after the break (Thursday afternoon and Friday; the Iraqi working week is Saturday to Thursday); thereafter, there were no symptoms or symptoms of lesser severity on other days of the week. The workers had been employed between 17 and 30 years at the same plant and had experienced symptoms between 1 and 18 years previously (Table I).

**Normal Subjects**

Thirty-one subjects within the same general age group as the byssinotic subjects (40 to 60 years) were city dwellers who worked at the College of Medicine at Baghdad. None had a history of exposure in dusty occupations. For comparative analysis, they were divided into nonsmokers (n = 12; average age, 46 ± 6 years), smokers (n = 9;
Table 1—Data from 29 Byssinotic Iraqis*

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Range (yr)</th>
<th>Mean (yr)</th>
<th>Range (Cotton, yr)</th>
<th>Mean (Cotton, yr)</th>
<th>Range (Symptoms, yr)</th>
<th>Mean (Symptoms, yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmokers</td>
<td>11</td>
<td>33-55</td>
<td>46±6</td>
<td>17-30</td>
<td>25±4</td>
<td>3-18</td>
<td>7±4</td>
</tr>
<tr>
<td>Smokers</td>
<td>11</td>
<td>42-58</td>
<td>45±5</td>
<td>20-30</td>
<td>26±4</td>
<td>1-18</td>
<td>8±6</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>7</td>
<td>40-62</td>
<td>54±7</td>
<td>20-29</td>
<td>24±4</td>
<td>2-10</td>
<td>6±3</td>
</tr>
</tbody>
</table>

*Grade 1 and grade 2 byssinosis.

average age, 50±6 years), and ex-smokers (n=10; average age, 50±6 years).

Condensed Polyphenol

Condensed polyphenol (polyphenol) was prepared as described by Taylor et al* from bracts of cotton plants (supplied by Dr. F. Sasser of Cotton Incorporated, USA). The yield of purified material from 100 g of dried bracts was about 2 g. Limulus assay and rabbit pyrogen testing showed no endotoxin activity. This was made up at 500µg/ml in saline solution prior to use and sterilized using a 220µ filter. All subjects were skin tested with polyphenol prior to challenge to eliminate any possible gross reaction.

Inhalation Challenge

This was carried out according to the method outlined previously.* The polyphenol at 500µg/ml was nebulized using an inspiron mini nebulizer and delivered to the subject via a loose-fitting face mask. After ten minutes, approximately 2 ml of material was nebulized, and analysis of particle size by Boyco airborne particle analyzer (Gellman Instruments Ltd) revealed that nearly all particles were less than 10µ, and most were less than 5µ.

The pattern of inhalation exposure was as follows: day 1 (Saturday), polyphenol; day 2 (Sunday), saline solution; or day 1, saline solution; day 3, polyphenol; controlled on a double-blind basis. The FEV1, and forced vital capacity (FVC) were determined before challenge and at 15 minutes, one hour, and two hours after challenge using a dry spirometer (Vitalograph). Subjects were also questioned for up to four hours after exposure regarding possible symptoms in general terms, and also whether they felt symptoms similar to those experienced at work. On the second and third days, they were asked about late symptoms that might have developed during the evening.

Results

Analysis of the groups with respect to smoking behavior is shown in Table 1. They provided very close groups in terms of average age, length of employment at the factory, and duration of byssinotic symptoms.

None of the 29 workers reacted to polyphenol to produce symptoms they had described in starting the week at the factory. Twelve of the workers voiced mild complaints. Five had vague symptoms of discomfort during nebulization (three with polyphenol and two with saline). Two had throat irritation (one with polyphenol and one with saline). Five complained of transient chest tightness (three with polyphenol and two with saline). It is of interest that ten of the 12 complained after the first nebulization. Clearly, it can be seen that there is little difference between polyphenol and saline solution when these byssinotic subjects were challenged according to a previously outlined procedure.10

Pulmonary Function

Inhalation Challenge. The mode of analysis chosen was to convert the readings at zero time, 15 minutes, one hour, and 2 hours into a mean value for FEV1, and FVC, an overall slope over the two-hour period, and a rate of increase or decrease of slope. The three indices are independent of each other and between them we can cover all of the most reasonable shapes for a time-response curve.14

The mean levels tended to be higher with polyphenol (FEV1, 2.77 L/sec; FVC, 3.67 L) than with saline solution (FEV1, 2.75 L/sec; FVC, 3.65 L), although the differences (FEV1, 0.02 ± 0.04 L/sec; FVC, 0.02 ± 0.02 L) are not statistically significant.

The slope of the curves over the two-hour period showed a slight decrease for polyphenol (FEV1, −0.005 L/hr) and a larger decrease for saline solution (FEV1, −0.027 L/hr); the difference was 0.022 ± 0.035, which is not significant. For FVC an increase was recorded for polyphenol of 0.037 L/hr and a decrease for saline solution of −0.029 L/hr (difference, 0.066 ± 0.069), again not significant. The rate of increase or decrease of the slope also did not differ significantly for polyphenol and saline solution.

Comparison of Pulmonary Function with Non-byssinotic groups. For this purpose the mean of the results at zero time (before polyphenol and saline solution) was used for the byssinotic subjects. All values for FEV1 and FVC were standardized to a height of 1.70 meters by dividing by the square of the man's height and multiplying by 1.7. The three smoking groups were treated separately.

The results are summarized in Table 2, which shows significant differences in both FEV1 and FVC between the nonsmoking normal subjects and byssinotic subjects and differences approaching but not quite reaching the 5 percent level of significance for the smoking group.

Table 2—Pulmonary Function in 29 Byssinotic and 31 Normal Iraqis*

<table>
<thead>
<tr>
<th>Group and Data</th>
<th>Normal</th>
<th>Byssinotic</th>
<th>Difference ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>FEV1, L/sec</td>
<td>3.82</td>
<td>3.24</td>
<td>0.58 ± 0.19</td>
</tr>
<tr>
<td>FVC, L</td>
<td>4.63</td>
<td>4.10</td>
<td>0.53 ± 0.26</td>
</tr>
<tr>
<td>Smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>9</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>FEV1, L/sec</td>
<td>2.96</td>
<td>2.50</td>
<td>0.46 ± 0.27</td>
</tr>
<tr>
<td>FVC, L</td>
<td>3.87</td>
<td>3.53</td>
<td>0.34 ± 0.25</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>FEV1, L/sec</td>
<td>3.28</td>
<td>2.54</td>
<td>0.73 ± 0.35</td>
</tr>
<tr>
<td>FVC, L</td>
<td>4.20</td>
<td>3.61</td>
<td>0.58 ± 0.37</td>
</tr>
</tbody>
</table>

*Values taken prior to inhalation challenge and height adjusted. Differences in results for nonsmokers and ex-smokers are significant at 5 percent level. Those for smokers approach but do not reach significance at 5 percent level.
byssinotic subjects vs smoking normal subjects. A pattern similar to the nonsmokers emerged for ex-smokers, although with the inclusion of one young normal subject with a high FVC and two older byssinotic subjects with very low FEV₁ and FVC, these differences could technically have arisen by chance. Adjustments for the slight differences in ages of the comparative groups did not alter the significance of the results.

**Discussion**

Although the study was originally designed to observe the effect of polyphenol on a small group of byssinotic subjects, the lack of response in the original group of eight symptomatic cotton workers (four non-smokers and four smokers) prompted a larger study with an extra 21 workers participating. None of the 29 men experienced symptoms; thus, we were unable to confirm the earlier results of Taylor et al. The polyphenol was freshly prepared, freeze-dried, and reconstituted just prior to use; it had lost none of its biologic activity, as determined by its capacity to nonspecifically precipitate protein. The nebulizer produced droplets, nearly all of which were less than 10μ in size, as determined by a Royco particle size analyzer; most were less than 5μ. The only obvious difference is in the ethnic groupings. All of our subjects were Iraqi, whereas those of Taylor et al, although not defined, were probably non-Iraqi.

Another feature that is the symptoms recorded by Taylor et al after inhalation challenge were not accompanied by change in the FEV₁, and it may be the case that this particular pulmonary function parameter is not the best measure of response to be used in studying byssinosis. As early as 1936, Prausnitz observed that all of the byssinotic patients questioned “insisted that their breathlessness was strictly inspiratory,” and no difficulty in expiration was noted. It is possible that measurement of deep inspiration may more readily differentiate byssinotic from nonbyssinotic subjects, thereby producing a reversal of the classic pattern as seen in asthma, with difficulty in expiration and less difficulty in inspiration. The pulmonary function results confirmed the findings in many other studies of a marked reduction in pulmonary function in smokers compared with nonsmoking individuals. This was exacerbated by the long-term effects of inhaling cotton dust (Table 2). The effects of the dust itself produced a significant difference when data on FEV₁ and FVC of nonsmoking byssinotic subjects and normal subjects were compared. A marked reduction in those two pulmonary function parameters occurred in the byssinotic subjects, although such results have been observed previously. Honeybourne et al recommended that the work on bronchial challenge with polyphenol should be repeated. This has been achieved; however, our results do not add in a positive manner to our knowledge of the etiologic agent in byssinosis. It may be the case that more than one agent in cotton dust should now be considered.

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**References**