studies, to evaluate the consequences of the observations made, in the same way as it seems difficult to understand the cause of the probable significance of light in relation to the concentration of choline in serum. It seems, however, that determinations for choline in serum might present a possibility of measuring—by a fairly simple method—the effect of light on the organism. Furthermore, the variations seem to be so large that a chemical method of determination might well be employed.

17038

The Pathogenicity of Bagasse, II. Effect on Rabbits of Prolonged Exposure to Bagasse.*

B. GERSTL, M. TAGER.† AND L. W. SZCZEPANIAK.

From the Central Laboratory of Pathology and Research, State Tuberculosis Commission, Hartford, Conn., and the Laboratory Service, Veterans Administration Hospital, Oakland, Calif.

The clinical and industrial health problems of Bagasse Disease have attracted increasing attention.[1-12] In spite of careful observation and biopsies on patients suffering of this disease, its pathogenesis remains obscure.[2]

Various factors have been suggested as etiologic agents: the particular composition of the fiber itself, fungi and micro-organisms attached to the fiber, and the high silica content. Allergic phenomena also entertained as a possible etiologic mechanism[3] seem, on the basis of experimental evidence,[4] less likely to be involved.

The problem of establishing whether the fiber itself, or the micro-organisms growing in abundance on it,[4,5] is responsible for the disease was approached experimentally by comparing the lesions produced by either untreated, autoclaved, or formalized bagasse.[4] In short term experiments a striking difference was observed. Rabbits developed a rapidly progressing, even fatal, disease after intravenous or intratracheal administration of fresh bagasse, while the autoclaved or formalized material produced only a foreign body reaction limited to the lungs. In the present report these experiments were extended in order to study the character of the lesions at longer intervals, to resolve the complex histopathology of the lesions into components that could be correlated with the various ingredients of bagasse dust, and to compare the morphology of the experimental lesions with that of the human disease.

Material and methods. For intratracheal insufflation, a procedure described earlier was employed.[4] The attempt to expose animals

* Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the author.

† Department of Bacteriology, Yale University Medical School; present address: Department of Microbiology, Western Reserve University Medical School, Cleveland, Ohio.

1 Anonymous, *J. A. M. A.*, 1948, 1050.

2 Lemone, D. V., Scott, W. G., Moore, S., and Koven, A. L., *Radiol.*, 1947, **49**, 556.

3 Castleden, L. I. M., and Hamilton-Paterson, J. L., *Brit. M. J.*, 1942, **2**, 478.

4 Gerstl, B., Tager, M., and Marinaro, N. A., *Arch. Path.*, 1947, **44**, 343.

5 Hunter, D., and Perry, K. M. A., *Brit. J. Industr. Med.*, 1946, **3**, 64.

6 Sonkin, L., Lipton, W., and Van Hoesen, D., *J. Industr. Hyg. and Tox.*, 1946, **28**, 273.

7 Gardner, L. U., *Am. Rev. Tuberc.*, 1920, **4**, 734.

8 Moore, M., *Arch. Path.*, 1946, **42**, 113.

9 Browne, C. A., *J. Am. Chem. Soc.*, 1904, **26**, 1221.

10 Hirsch, E. F., and Russel, H. B., *Arch. Path.*, 1945, **39**, 281.

11 Sodeman, W. A., and Pullen, R. L., *Arch. Int. Med.*, 1944, **73**, 365.

12 Koven, A. L., *Am. Rev. Tuberc.*, 1948, **58**, 55.

TABLE I.
Rabbits Exposed to Bagasse in Dusting Chamber.

Rabbit No.	No. of hours exposed	Duration of exposure, days	Day after last exposure that animal died (d) or was sacrificed (s)
296	270	108	11th (s)
297	270	108	21st (s)
347	30	6	1st (d)
356	72	15	1st (d)
359	90	15	1st (d)
362	180	44	24th (s)
352	30	6	30th (s)
363	180	44	36th (s)
348	54	12	36th (s)

to a continuous and somewhat controlled flow of dust for days or weeks met with difficulty. An arrangement, similar to that recommended by Sonkin *et al.*[6] proved unsuccessful because of the hygroscopic bagasse powder rapidly becoming sticky and plugging the jet. Shaking a large amount of bagasse and maintaining the dust in the air by means of a fan or blower resulted in unequal distribution in the dusting chamber. Finally, resort was taken to a modification of the relatively simple method employed by Gardner.[7] Bagasse was finely shredded in a Waring blender and strained through a wire gauze sieve, 150 meshes per inch. Dusting was carried out for various periods during the day time. (See Table I). Approximately 15 g of bagasse were expended during 6 hours of dusting. The animals with their fur thickly coated with dust were then replaced in their cages.

To secure ash, bagasse was kept in an electric furnace at 850°F, with air blown into the oven to insure oxygenation. The resulting ash was ground in a glass homogenizer. The average yield was 5.74%. Hunter and Perry[5] as well as Koven,[12] reported 3 to 4%. The silica content of the ash was 43.34 and 43.05%.‡ The ash of 10 g bagasse, ground with the addition of saline, was made up to 90 cc. Each cubic millimeter contained approximately 35,000 particles. Its total content of solid particles, estimated volumetrically after centrifugation, was twice that of suspension of bagasse.[4] The mineral content, however, was about 10 times that of the suspension of bagasse fiber.

An extract of the resinous substances was obtained by refluxing 5 g of shredded bagasse with 100 cc of petrol ether or benzol for 10 hours. The solvent was evaporated from the filtrate. The residue suspended well in 15 to 20 cc of water, to which 2 drops of monoethanolamine had been added.

Results. A. Intratracheal insufflation of bagasse. Rabbits studied at 20 and 35 days after the intratracheal insufflation of 10 ml of suspension of fresh bagasse showed frequent and large pneumonic lesions, with the alveolar exudate predominantly monocytic in character, though polymorphonuclears were numerous in places. The interstitium was infiltrated by small and large round cells. Fibroblastic proliferation was noted at the periphery of several lesions. Other lesions were of a foreign body granulomatous type and composed of multinucleated giant cells and mononuclears grouped together by bundles of fibroblasts. Cytoplasmic defects of irregular shape were often seen in the giant and mononuclear cells, but birefringent bodies could be identified under polarized light only in a few instances. From the pulmonary lesions of one of the rabbits (35th day) an Aspergillus was isolated on culture. In some sections radiate bodies (Moore[8]) could also be identified. Rabbits treated similarly with autoclaved or formalized bagasse and studied at identical intervals revealed lesions composed of one to several multinucleated giant and mononuclear cells, both showing cytoplasmic vacuoles. Slight fibroblastic pro-

‡ These values were obtained on a single sample and carried out by Dr. Carl Tiedcke, New York City.

liferation and a few small round cells were seen at the periphery of some of the lesions, rendering them granuloma-like. There was little difference between the morphology of these lesions and those observed at 10-day intervals.[4]

B. Effect of ash and resins of bagasse. For the purpose of differentiating the effect of minerals present in bagasse from that of the fiber as a whole, 2 rabbits received each 10 ml of ash suspension, as described under Materials and Methods, by the intratracheal route, and were sacrificed 35 days later. Microscopic sections revealed occasional foreign body giant cells, both single or in small groups, in the interstitial pulmonary tissue. Some irregular shaped cytoplasmic defects in these cells corresponded, under polarized light, to double refractile bodies. In the pulmonary sections of one of the animals two small granulomatous lesions, composed of a few multinucleated giant cells and fibroblastic proliferation were noted; and in a section of the other animal a few alveoli plugged by young connective tissue were noted. (Fig. 1).

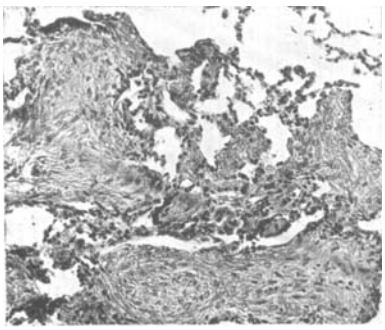

FIG. 1.
Rabbit 328. Treated intratracheally with ash of bagasse; 35th day. Lung. Masson strain. ×75.

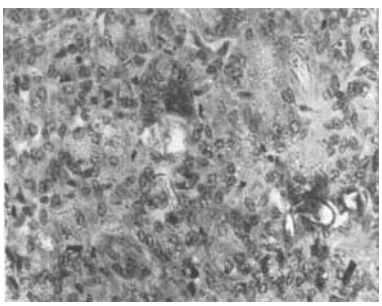

FIG. 2.
Guinea pig. Intraperitoneal injection of ash of bagasse; 90th day. Peritoneal tissue. H. & E. stain. ×165.

Intravenous administration of similar material in repeated small doses, totaling 8 ml produced occlusion of numerous capillaries by multinucleated giant cells which had formed around irregular shaped, sometimes highly refractile, foreign bodies. Similar giant cells were also found occluding lymphatics. This type of lesion was observed at 3 and 4 day intervals as well as at 20 and 35 days. In the latter group they differed only by being less numerous and by the occasional presence of a few small round cells or slight fibroblastic proliferation around the giant cells. The pulmonary changes in two rabbits, treated similarly and sacrificed at 55 days, were almost identical to those of the aforementioned ones. It is noteworthy that the other organs of these animals were free of lesions.

Three guinea pigs each received intraperitoneal injections of 2 cc of the ash suspension, and were sacrificed 90 days later. Two of them revealed granulomatous lesions attached to the visceral and parietal peritoneum, and composed of numerous large giant cells, mononuclears, and slight fibrous tissue proliferation (Fig. 2). Numerous variously shaped foreign bodies were within cytoplasmic vacuoles of these cells. None of these lesions resembled those seen in experimental silicosis.

The extent and number of lesions, as well as the intensity of the cellular reaction in the rabbits that received ash suspension were strikingly less than in the animals treated with autoclaved or formalized bagasse, although the mineral content of the material administered was a multiple of that in the former group.

Browne,[9] in an early investigation of the composition of bagasse, pointed to its high resin content. Resins are not innocuous substances, although the literature on this subject is scanty (Hirsch and Russel[10]). An attempt was, therefore, made to arrive at an appraisal of the biologic properties of the resins present in bagasse. Extracts, prepared as stated under Methods, and suspended in ethanolamine water were administered intra-

tracheally so that each of three rabbits received the total from 2.5 g of bagasse. The animals were sacrificed on the 35th day. A moderate number of multinucleated giant cells of the foreign body type were seen in the interstitial tissue of the lungs. Some of them contained large, irregularly shaped, foreign bodies which did not rotate the beam of polarized light. There was no other cellular reaction.

C. Exposure to bagasse dust. Animals were exposed to dust for periods indicated in Table I. For dusting rabbits 296 and 297, various mechanisms including the jet devised by Sonkin *et al.*[6] were employed. Only for the last 10 hours of exposure were these animals kept in the dusting chamber used for all other animals listed in Table I. Between the two animals there was a striking difference in type and extent of lesions. A few alveoli containing a mononuclear exudate were the only changes seen in rabbit 297. Although rabbit 296 was sacrificed 10 days later, its necropsy revealed extensive acute inflammatory changes. In the lung, numerous alveoli were filled by a polymorphonuclear and monocytic exudate with some of their nuclei showing pyknosis and karyorrhexis. Disintegrated cells were also noted in the perivascular lymphatic tissue. Foci of necrosis were present in liver and spleen.

Three animals, kept in the dusting chamber as indicated (Table I), died after 30, 72 and 90 hours of exposure. (Rabbits 347, 356 and 359). It is noteworthy that their exposure mates survived for a prolonged period. The microscopic preparations of the first rabbit revealed a hemorrhagic pneumonia; those of rabbit 359 revealed fairly numerous mononuclear cells in alveoli and in distended lymphatics. Small foreign bodies were seen in many exudate cells. Two healing myocardial infarcts may have accounted for the early death of this animal. The sections of rabbit 356 revealed an extensive pneumonic process with the exudate being polymorphonuclear in places, in others predominantly mononuclear. Many bronchioli were plugged by a similar, sometimes disintegrated, exudate. Occasional organization of the bronchiolar and bronchial exudate was found (Fig. 3). Multinucleated giant cells were infrequent. In addition to numerous small variously shaped foreign bodies there was noted an occasional club-shaped body, continuous with a fragment of a mycelial-like structure (Fig. 4). Frequently groups of large mononuclears with a foamy cytoplasm were seen both in alveoli and interstitium. Similar changes were also responsible for some polyp-like protrusions of the interstitium into the alveolar lumina. Some of the alveoli were lined by a cuboidal metaplastic epithelium.

Four animals were exposed to bagasse dust from 30 to 180 hours, and sacrificed at intervals from 24 to 36 days after the last exposure. These animals, because of the approximating interval, as well as the similarity

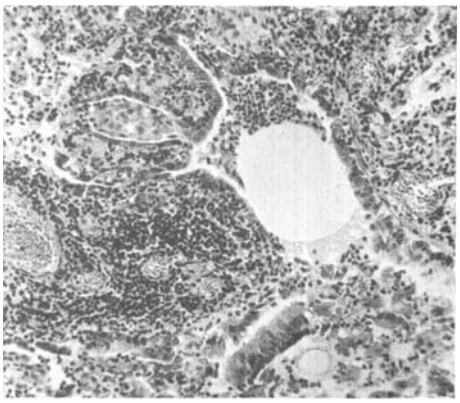

FIG. 3.
Rabbit 346. Exposed to bagasse dust for 72 hours. Died. Lung. H. & E. stain. ×100. Bronchioli plugged by exudate. Interstitial infiltration by inflammatory cells.

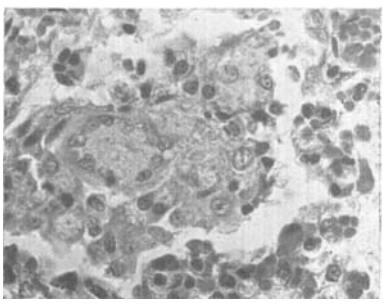

FIG. 4.
Rabbit 356. Lung. Masson stain. ×425. Spore and mycelia-like structure; foreign body giant cell.

of lesions, may be considered together. There were many alveoli in groups of 3-20 which were filled by closely packed large round or polygonal cells, some of which could be identified as mononuclear phagocytes. Their cytoplasm contained brown dust-like material and also birefringent rods. A few multinucleated giant cells of the foreign body type and occasional polymorphonuclears were present. There was no necrosis. In pulmonary sections of rabbit 363 there was a marked thickening of the interstitium by monocytic infiltration and fibroblastic proliferation sometimes forming protrusions (Fig. 5) similar to those observed in rabbit 356.

Three guinea pigs (337, 338 and 339) were also exposed in the dusting chamber for a total of 77 hours over a period of 18 days, and sacrificed 10 days later. The short interval was selected in order to observe any acute inflammatory changes which might have subsided at a later date. In the pulmonary sections some alveoli and bronchioli contained an exudate of large vacuolated mononuclear cells and an occasional polymorphonuclear. Elsewhere, large multinucleated giant cells were seen. Numerous foreign bodies were present within cells of both types. When compared with the lesions of rabbits exposed for a similar length of time, the almost complete absence of an acute inflammatory response in the guinea pig was striking.

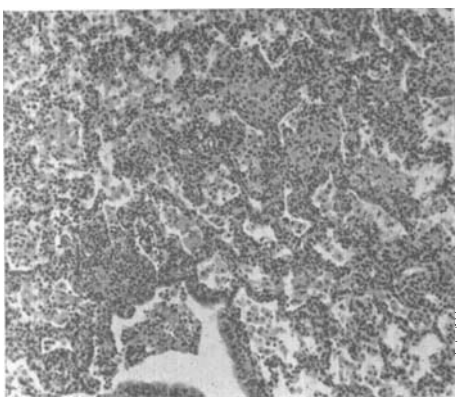

FIG. 5.
Rabbit 363. Dusted 180 hours, 36th day. Lung. H. & E. stain. ×60. Marked interstitial infiltration.

Discussion. The striking difference between lesions resulting from the administration of live bagasse, and those from bagasse dust with all living matter killed by either autoclaving or formalizing, as described first in short time experiments, was also manifest in animals kept alive for longer intervals. This is suggestive evidence that some living matter attached to the bagasse fiber, in itself an injurious agent, enhances the effect, and produces an additional pathologic change. The bagasse fiber, without living matter, elicits a long-standing foreign body reaction. Bagasse dust in the native state, when inhaled or insufflated, calls forth, in addition to the foreign body granuloma, an acute inflammatory response which, in view of its presence many days after the exposure, allows no other interpretation than that self-propagating microorganisms act upon the tissue of the host. For the rabbit, an aspergillus seems to be the pathogenic agent. It could be recovered from the pulmonary lesions 35 days after intratracheal administration of bagasse dust. This finding would support the theses of other authors[3,5,12] that fungi play an important part in the pathogenesis of the human disease.

Two components of the bagasse fiber could be demonstrated as being responsible for a long persistent tissue reaction, although the latter is of minor degree. One of them, the mineral particles, is of interest for the high silica content. But none of the lesions produced by the mineral ash resembled those of human or experimental silicosis. This would bear out the assumption of several authors that human Bagassosis is not caused by the high silica content of the bagasse fiber. The resins similarly contribute to the persistence of the lesions, as evidenced by the presence of giant cells 35 days after intratracheal insufflation. It also could be established that the resins, at least in their extracted form, do not contribute to the birefringence of the bagasse particles.

The most interesting part of the investigation was the effect of bagasse dust when inhaled by animals under conditions simulating those under which the human disease is acquired. It was soon apparent that some of the animals reacted violently to the dust with

instant and protracted cough, and developed a rapidly progressing pneumonia. The complex morphology of the lesions found in these animals includes hemorrhagic pneumonia, bronchiolitis, with plugging of passageways by cellular exudate and debris, interstitial thickening by exudate and fibroblastic proliferation, as well as of foreign body reaction. On the other side are the animals that offered a higher resistance, possibly aided by elimination of some particles by the upper respiratory channels. These animals probably would have survived indefinitely and demonstrated the ability to resolve gradually the lesions which they undoubtedly harbored at an earlier date.

Although these two groups of animals represent an interesting parallel to the selective morbidity among workers in the bagasse industry, a comparison of the morphology of the human with that of the experimental disease is hindered by the scarcity of human material available.§

Of the 2 cases that came to autopsy (Sodeman,[11] Hunter and Perry[5]) only one has been reported so far. Large spicules, as illustrated in Sodeman's case, could not be found in the tissue of animals that were exposed to the dust. Apparently particles of that size could not pass the smaller respiratory lumina of the animals. Other characteristic features are interstitial fibrosis, bronchiolitis,[5] and the presence of numerous large foamy alveolar cells filling the alveolar spaces.[11]

In the experimental lesions interstitial fibrosis was scanty. This may be due to a greater resolving power of the animal tissue or to the fact that the more susceptible animals succumbed too early (6 and 15 days) to have developed extensive fibrosis. Fibroblastic proliferation, however, was observed. Large foamy exudate cells and plugging of the bronchi were the features whereby the experimental most closely resembled the spontaneous disease.

The character of the lesions in the guinea pig differed strikingly from that in the rabbit. These represented a response to foreign bodies and lacked the acute inflammatory and progressive component, and are apparently due to the fact that the guinea pig is not susceptible to the organisms associated with bagasse.[4,5]

Conclusions. These experimental and comparative studies permit the conclusion that the inorganic part of the inhaled bagasse dust produces a long-standing tissue reaction which is primarily a foreign body response. It is unlike silicosis and amenable to healing by resolution. Superimposed on these lesions there occurs in those animals which are more susceptible to the causative microorganisms acute bronchiolitic and pneumonic changes which, if sufficiently extensive, may cause death of the animal. A similar combination of etiologic factors seems to be the most plausible explanation of the complex picture of human bagasse disease.

Summary. Bagasse irrespective of the route of administration, produces a complex and frequently progressive inflammatory reaction of the exposed animal. By employing native, in contrast to autoclaved bagasse and its extracted resins and minerals, it could be demonstrated that the fiber itself calls forth a long-standing foreign body reaction which is amenable to healing. The progressive inflammatory reaction and death of the animal, however, are due to microorganisms attached to the bagasse. In the instance of the rabbit, aspergilli are the pathogenic agents. The points of similarity of the experimental lesions to those observed in humans are discussed. None of the lesions resembled silicosis.

§ Dr. W. A. Sodeman kindly made available several slides of the pulmonary lesions of the case observed at Tulane University.

I wish to express sincere thanks to the Hospital Photographic Laboratory, Letterman General Hospital, San Francisco, for preparing these microphotographs.