EXPERIMENTAL BRONCHOPNEUMONIA

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In the investigation of pneumonia to the present day there exist many unsolved questions. One of the reasons for this is the absence of a reliable method by means of which experimental pneumonia can be produced so that the natural course of the disease could be duplicated.

Most workers attempted to reproduce croupous pneumonia, using as a basis the allergic character of the illness [1, 2, 4, 5, 10, 14, 15, 16, 17, 18, 19].

When attempts were made to reproduce bronchopneumonia, the successes were few. The attempts in this direction can be divided into two groups.

In the first group are experiments affecting the various divisions of the nervous system which upset the circulation by reflex means and so produce a consequent pneumonia. Thus, A. D. Speransky [10] established that irritation in the subcollicular regions of the brain leads to trophic lung disturbances against which background pneumonia may develop. A. V. Tonkikh [11] described pneumonia following crushing of cervical sympathetics (in cats) or sectioning of vagi (in cats and dogs). Analogous results were obtained by A. M. Chernukh by injecting turpentine along neural sheaths of vagi (in rabbits) [13] and I. P. Dolgachev [3] when applying radon to the upper cervical sympathetic node (rabbits, cats). However, many of the above experiments failed to have adequate pathologic and microbiologic controls.

As was shown by B. I. Monastyrskaya and Ya. Ya. Sokolskaya [6, 7, 9] action on the vegetative nervous system disturbs the circulation in the lungs which can exist a long time without producing inflammatory changes. Only in occasional instances with bilateral vagal section or stimulation of the sympathetic nodes will aspiration pneumonia be an end result.

In the second group of models are attempts to reproduce pneumonia by affecting lung tissues directly by way of intratracheal infection of the animals with live bacterial cultures. This was the method employed by Z. L. Golsand and A. V. Tsinzerling [12] on rats and D. S. Sarkisov and L. Ya. Ebert on cats [9]. The first infected rats with Friedlander bacilli in a 6% mixture with gum arabic. The microbial culture was introduced into the animal tracheal lumen, first incising the overlying skin while the animals were under ether narcosis. As a result the animals developed lobar or large-focus bronchopneumonia. In their experiments there was a combination of bacteriologic and morphological studies.

However, this method has been successful in rats only, and then only if Friedlander bacilli are used. This is a limiting factor when using the method of Golsand and Tsinzerling.

D. S. Sarisov and L. Ya. Ebert introduced intratracheally into cats under ether and chloroform narcosis cultures of Staphylococcus aureus in a 16% suspension of polyvinyl alcohol. This alcohol was chosen for the prolonged fixation and more even distribution of the organisms through the lungs. In animals infected by this procedure isolated small foci of pus inflammation arose around the bronchi.

A serious inadequacy of both of the just described methods is the use of gum arabic, polyvinyl alcohol and inhalation narcosis. These substances undoubtedly have an additional injurious effect upon the lung tissue so that the pneumonia produced is in a large degree quite different from those occurring spontaneously.

Taking all the above into consideration, in 1954 we worked on rabbits attempting to produce pneumonia by intratracheal injections of cultures of B. enteritidis gartneri the original culture having been recovered from a spontaneous case developed by a rabbit.

The cultural characteristics of the microbe are indicated in the table.

Morphology	Motility	Biochemical response						Titer of agglutination reaction with sera				Lethal for mice
		Glucose	Lactose	Mannose	Saccharose	H_2S	Indole	Abdominal typhus	Paratyphoid B.	Breslau	Gartner	
Gram-negative rod	+	KG	0	KG	0	+	0	1:1,600	1:800	1:200	1:6,400 1:12,800	+

EXPERIMENTAL METHODS

Intratracheal introduction of the microbes was performed by using a needle without any previous incision or narcosis. The animals withstood the manipulations quite well. First the skin on the anterior surface of the neck was clipped and shaved as thoroughly as possible. The rabbit was then bound to a board so that the head fitted into a hollow in its upper portion while the fore paws could be spread as far apart as possible and also downward. The skin of the area to be injected was sterilized with iodine and alcohol. The board was tilted at about 40-45° of head elevation and the head was also tilted backwards. In this position it was a simple matter to palpate the cartilage of larynx and trachea. The skin was first pierced by the needle held in exactly the midline. Then the fingers of the left hand fixed the trachea which was pierced by the neeedle about 0.5-1 cm below the larynx. At the moment of entry which was always from above down the needle was held at a maximal angle from the surface of the skin. This was done to avoid injuring the posterior surface of the trachea. The puncture was done with a sterile needle attached to a hypodermic syringe half filled with physiological saline so as to avoid infecting any surrounding tissues at the moment of entry.

After the puncture we made certain that the needle tip was in the tracheal lumen. This was readily confirmed by pulling back on the piston of the syringe and obtaining air bubbles. Then the syringe holding the saline was replaced with the one having the required amount of infecting material, and the material was introduced quite slowly so as to minimize a possible cough reflex.

The infecting material was a suspension of B. enteritidis gartneri in physiological saline. From a one day agar culture a mixture was prepared with 100 million microbes per $^1/_2$ cc of physiological suspension (using optical bacteriological standards). As indicator which was to aid in judging the spread of the suspension through the lungs, Trypan blue was added (0.5% suspension). Experiments proved that this addition did not affect the pathological alterations.

EXPERIMENTAL RESULTS

Of 20 rabbits infected by the above method in the final confirmatory series, all developed pneumonia. One only died on the 2nd day after being infected; 11 rabbits were sacrificed on the 7th day; 1 died on the 17th; 4 were sacrificed on the 20th day and 2 -- on 30th day after the infection.

Daily blood cultures taken from the rabbit ear veins were consistently negative. This proved that the quantity chosen by us was sufficient to produce infection but did not produce bacteremia.

At autopsy all the animals were seen to have extensive pneumonic areas, sometimes involving an entire lobe or almost an entire lung (Fig. 1).

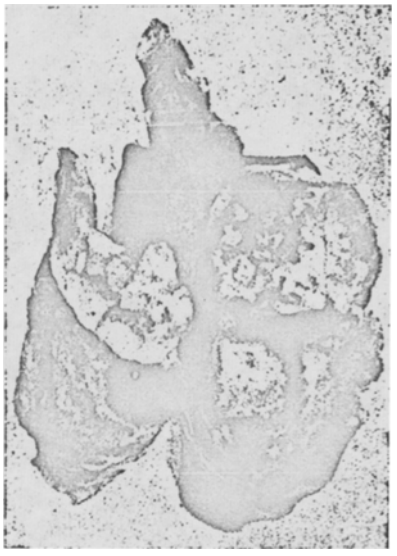

Fig. 1. Extensive areas of pneumonic involvement in both lungs 7 days after introduction of the infection.

The affected lung was much increased in volume, solidified, red in the first days, later mottled with yellow-grey areas and, when bluing was employed, of a bluish grey color (Fig. 2). The sections of the affected lung tissues had the same colorations and a finely granular surface from which steamed a thick, cloudy fluid.

The microscopic picture was that of bronchopneumonia (Fig. 3).

When the animal died in the first days after being infected, the alveoli had a serous exudate with an admixture of erythrocytes. Later, by the 7th day, the foci of consolidation had many alveoli and small bronchioles filled with exudate having pseudo-eosinophilic leucocytes, macrophages, septal cells and erythrocytes. In places, fibrin strands could be seen. Within the exudate the microbes were easy to see as they stained intensely with hematoxylin and were principally extracellular. Most of the cells within the exudate were in a state of necrobiosis and fragmentation. In the protoplasm of the macrophages were seen fragments of nuclei and eosinophilic granulation from the disintegrated leucocytes, microbes and granules of Trypan blue. The presence of the bluing in the alveoli indicated that the microbes introduced into the trachea had been able to reach the alveoli directly. Within the bronchi, the exudate consisted principally of leucocytes, cells of bronchial epithelium, macrophages, erythrocytes and microbes all mixed together with mucus. In the peribronchial tissues were observed lymphangeitis and hyperemia of the peribronchial lymph nodes. In places, within the lumen of the

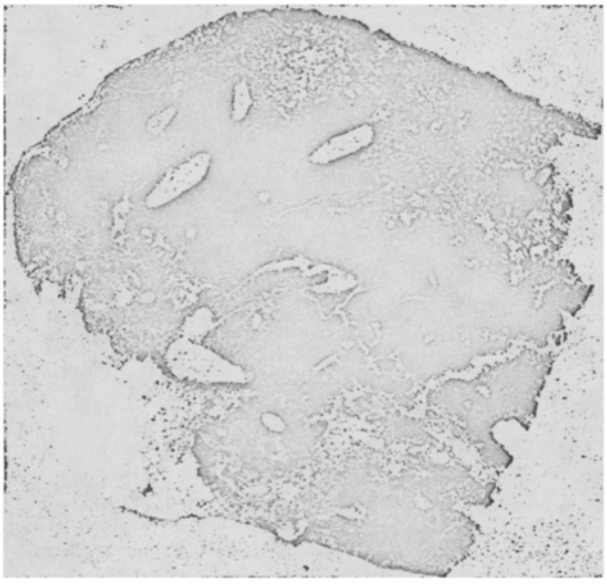

Fig. 2. An area of bronchopneumonia. Total section through the lower lobe of a lung on the seventh day after infection (stained with hematoxylin-eosin).

lymph vessels and within the lymph nodes, there were seen granules of the Trypan blue. In addition, there were lung areas with evidences of disturbed circulation (hyperemia, stasis, erythrocytic diapedesis with perivascular and peribronchial hemorrhages, edema of the stroma and alveoli).

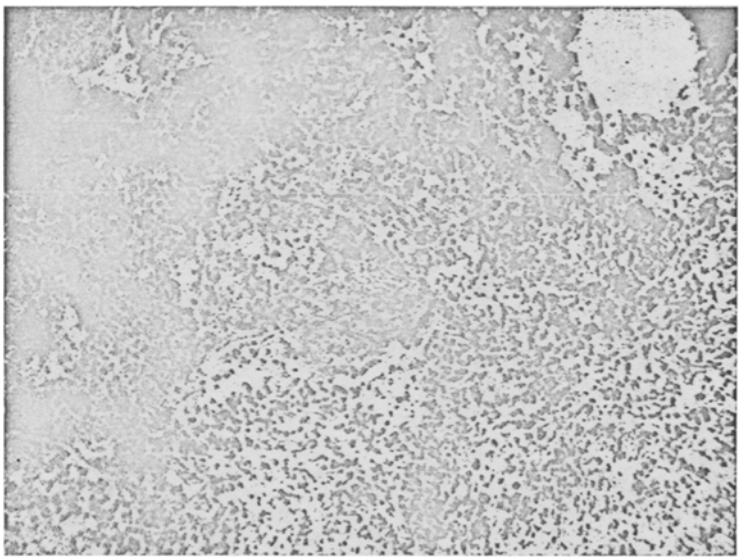

Fig. 3. Total section through the lower portion of the lung on the seventh day after infection. Alveoli of the lungs are filled with a catarrhal exudate with a large admixture of leucocytes; peribronchitis (hematoxylin-eosin stain, high magnification).

By the 17th-20th day after the infection, the pneumonic areas were much smaller than at the height of the infection on the 7th day. Microscopically, there were seen areas of atelectasis, a moderate number of leucocytes and many macrophages in the exudate. By the 30th day the inflammatory residues in the lungs were inconsequential.

All specimens taken at autopsy from the heart, spleen and gallbladder with one exception only were sterile. The rabbit that died on the 2nd day had pure culture of B. enteritidis gartneri recovered from specimen taken from its spleen. Alongside of this, all rabbits sacrificed between the 7th and 20th days had demonstrable bacteria in the lungs. By the 30th day the bacteria could no longer be obtained from the lungs either.

Thus, we have developed a completely reliable method for producing in rabbits a bronchopneumonia not associated with introduction of foreign bodies (such as plastic substances, gum arabic and agar) or the use of narcosis, not provoking bacteremia and assuring an adequate life span for the infected animals. Finally, our method does not demand the use of an unusual strain of microbe. The B. enteritidis gartneri we used is seen in spontaneous pneumonias and, therefore, is obtainable for experimental purposes.

SUMMARY

An experimental method for producing pneumonia in rabbits is presented. A culture of B. enteritidis gartneri was obtained from a rabbit having developed the disease spontaneously. This organism was used to reproduce the disease in 100% of the animals inoculated intratracheally.

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* In Russian.

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