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EARLY REACTION OF THE ORGANISM TO THE INTRODUCTION OF INORGANIC CORPUSCLES

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Almost as old as histopathology itself are our endeavours to ascertain the behaviour of the organism towards bacteria and towards corpuscular elements of a corresponding size.

As to inorganic substances, the majority of studies are concerned with industrial injuries, but a great number of experiments have been made with a view to gaining information about the capacity of the organism to phagocytize matter foreign to it.

The lung, an abundantly vascularized organ which is rich in macrophages and in direct contact with the outside world, has proved to be eminently suitable for investigations of this kind; last but not least because of the elective function it fulfills whenever corpuscular elements penetrate the organism.

Research workers have been faced for a long time with a number of ever recurring questions to which no unanimous reassuring answers have yet been forthcoming. In what manner do differently sized granules of certain substances pass into the organism through the lung, and vice versa? Is the storage of particles in the lung, and their elimination by it, affected differently according to their intravenous or intratracheal introduction to the organism? Is it the lung where the extraneous substance and the organism meet for the first time, and what is the organism's first reaction to the encounter?

Not only are the answers given to these questions not unanimous, but frequently they are contradictory. Undoubtedly, this is in part due to differences in the substances studied and the experimental conditions applied. The chemical diversity of the dyes and the differences in the size of the corpuscles assert themselves the more, the later the changes they cause come to be examined. By occluding vessels, the larger corpuscular elements alter parenchymal permeability at an early stage; chemical agents induce more or less grave lesions in organs; even intratracheally given saline damages epithelial cells in the respiratory tract by causing vacuoles to arise.

One of us (Fodor) has been engaged for a number of years in experiments concerned with the aetiology of syphilitic mesaortitis and arteriosclerosis. In one experimental series iron particles as Spirochaeta models, were injected intra-

venously. They differed in size from the coarse particles of reduced iron (10 to 30 μ and more in diameter) to such granules in much more uniform compositions, of which the finest measured as little in diameter as 200 Å. Dogs weighing from 5 to 8 kg were used as test animals, and were given considerable quantities (from 0,20 to 1,0 g) of iron in each experiment. In a simple suspension, the iron was injected into the jugular or femoral vein. Utilising the magnetic field of an ophtalmologic magnet, it was endeavoured in one group of animals, in the first 24 hours after the injection, to cause the differently sized iron particles to follow a certain course and to accumulate at certain sites in the organism. It is only for the sake of completeness that we report this attempt, since after a few months the storage conditions of the corpuscles were found to be the same in the organism irrespectively of whether a magnetic field had been applied or not.

Lasting for several weeks or months, these experiments furnished reliable information concerning the sites and mode of storage ; in sections prepared from the organs of animals killed with chloroform, the iron accumulations and the changes consequent upon them were distinctly recognisable.

Refraining from a detailed discussion of the results of these experiments, the striking feature, nevertheless, merits mentioning that despite the relatively grave organic lesions the dogs gained weight considerably during the introduction of iron, and that, apart from the vascular changes expected, iron accumulation was the most marked in cells belonging to the reticulohistiocytic system (RH) or performing facultative RH function. Equally conspicuous was the participation of the lymph paths in the transportation of iron (Fig. 1 B) ; especially replete with iron particles were the pulmonary lymphatics. Further conspicuous phenomena were the thickening of the alveolar septa (Fig. 1 A), the accumulation of iron in the basal layer of the pleura (Fig. 1 C), the saturation with it of the Kupffer cells (Fig. 1 E), its presence in the glomeruli of the kidney (Fig. 1 F) and, finally, the fact that while the proximal tubules contained hardly any iron, the peripheral ones were crowded with iron particles. The splenic parenchyma, too, contained many such granules.

Having encountered no similar findings in the literature it was deemed important to continue the investigation of some of these phenomena, first of all by way of acute experiments.

Methods

The animals used in our experiments were divided into groups of six, each containing four albino mice of an average weight of 20 g, and two guinea pigs of 250 to 300 g, originating from the same laboratory strains. A magnetic iron alloy containing 28 per cent nickel, and composed of minute particles of the same size and nature, was selected as the best medium to be followed up and demonstrated in the organism, and to study the latter's storing, eliminating, and other functions. This medium was reduced to powder, and particles of submicroscopic size to 10 μ were used for the mice, and of 10 to 20 μ for guinea pigs. They were introduced partly intratracheally, and partly intravenously. When administered intratracheally, the total dosage amounted to about 1 mg/kg which was given to the animals either in a dry

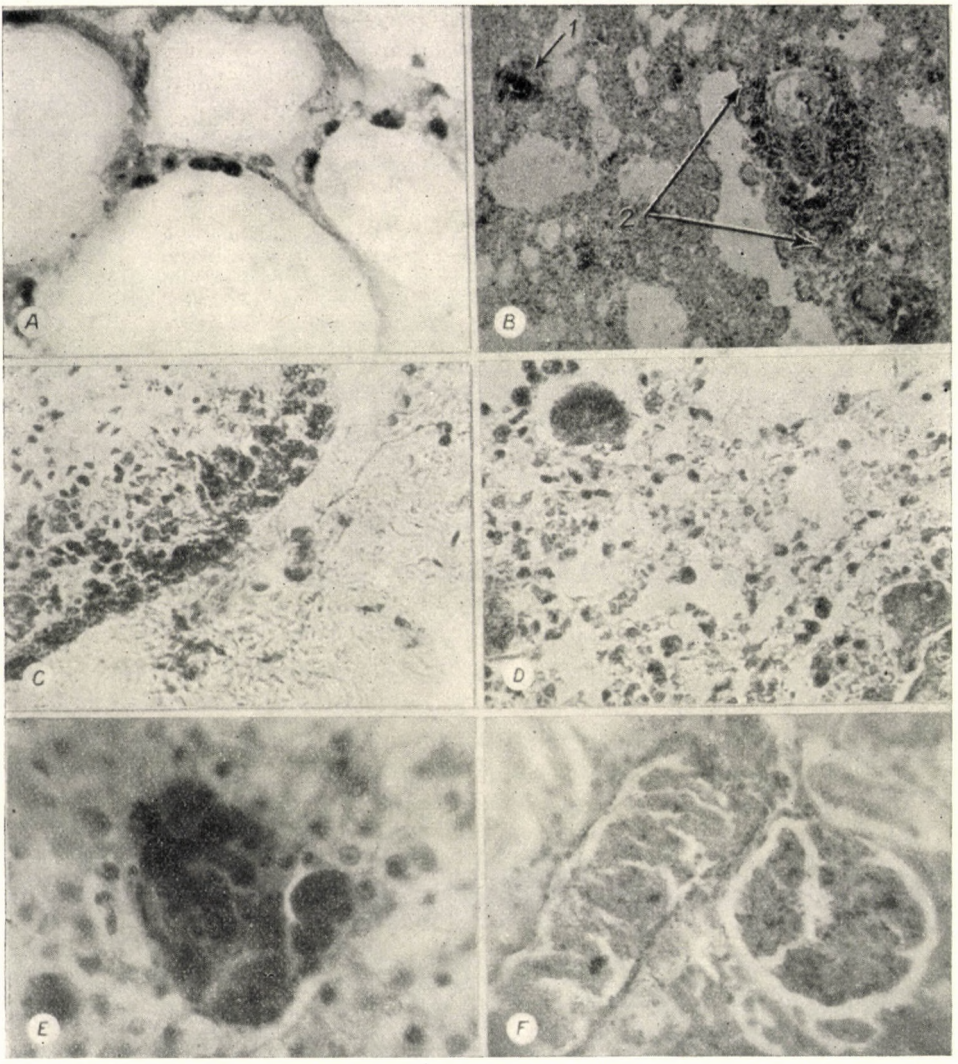

Fig. 1. Iron particles in dogs in protracted experiments

A. in alveolar cells,
B. 1) in the pulmonary lymphatic vessel; 2) perivascular storage; interstitial thickening,
C. subpleurally accumulated phagocytes; thickened pleura,
D. in giant cells,
E. in the liver,
F. in the kidney (Prussian-blue stain)

s tate or suspended in 0,1 ml of physiological saline ; when administered intravenously, the total was about 12,5 mg/kg and given in 0,1 ml of physiological saline. While the smaller particles passed through the pulmonary capillaries unobstructedly, the larger ones stuck there and formed clots. By cutting the medulla oblongata in ether anaesthesia, a group of six animals was each killed and autopsied immediately, after 1, 2, 3, 5, 8, 10, 15, 20, 60 minutes, and 24, 48, 72, and 120 hours, respectively. Fixing was effected by means of formalin and Maximov's fluid. In one part of the animals the fixing fluid was introduced through the trachea, reexpanding the lung in a chamber of negative pressure. In the earlier experiments (prolonged ones carried out in dogs), the Prussian-blue reaction had been used, but as in the organism, particularly in the lungs, of some animals iron can be demonstrated to be present even if none is introduced from without, the procedure was not held to be sufficiently conclusive for the present purposes. The use of a powerful magnet appeared to be much more suitable: the stained and freshly covered histological sections were first studied in detail, then placed in a magnetic field, thus forcing the iron particles to cluster from wherever they happened to be. The separation of granules of various origin microscopically observable in certain cells of healthy animals, refers to the macrophages of the lung: they failed to react to magnetic force. The sections were stained with haematoxylin-eosin and Van Gieson's stain ; the results obtained from a staining with Prussian-blue were taken into account only in connection with the prolonged experiments made on dogs.

In that series of experiments in which iron was introduced by means of intravenous injections, the following observations were made.

Particles between 5 and 15 μ in size usually stuck in the capillaries and precapillaries of the pulmonary circulation. Piling up, they formed emboli which remained in the lung for days, even months, and gave rise to early pathological lesions. The vascular and alveolar walls became highly permeable. As early as two minutes after the injection occasional detached macrophages could be observed on the epithelium of the bronchioles. On the introduction of 0,1 ml of the substance, in the primary and secondary bronchial branches the particles larger than 5 μ became visible outside the cells, not later than 5 minutes after the injection. The smaller particles filled up the macrophages.

For days the 1 to 5 μ particles moved freely in the pulmonary and systemic circulation. Hardly any of them were phagocyted by polynuclear leucocytes during the first few minutes, but thereafter phagocytosis began, to reach its peak at the 15th minute.

Following the path of the circulating particles, some of them were observed to pass through the vessel walls as early as the 90th second after their introduction, and to distribute evenly over the lungs, in contradiction to the findings of other authors.

Having reached the lung, some of the particles passed through the alveolar walls and escaped to the outside world through the bronchi (Fig. 2 C). Others began to be stored up with almost incredible rapidity, two minutes after injection, principally in the septal cells and in the histiocytes of the capillary walls and the walls of the bronchial arteries (Fig. 2 B). This process took the first 10 to 20 minutes to complete. So arranged, the particles remained visible for several days.

After the 5th minute proliferation of the mesothelial cells of the pleura set in.

As from the 15th minute the particles, and a little later the phagocytes loaded with them, began to escape through the visceral pleura as well.

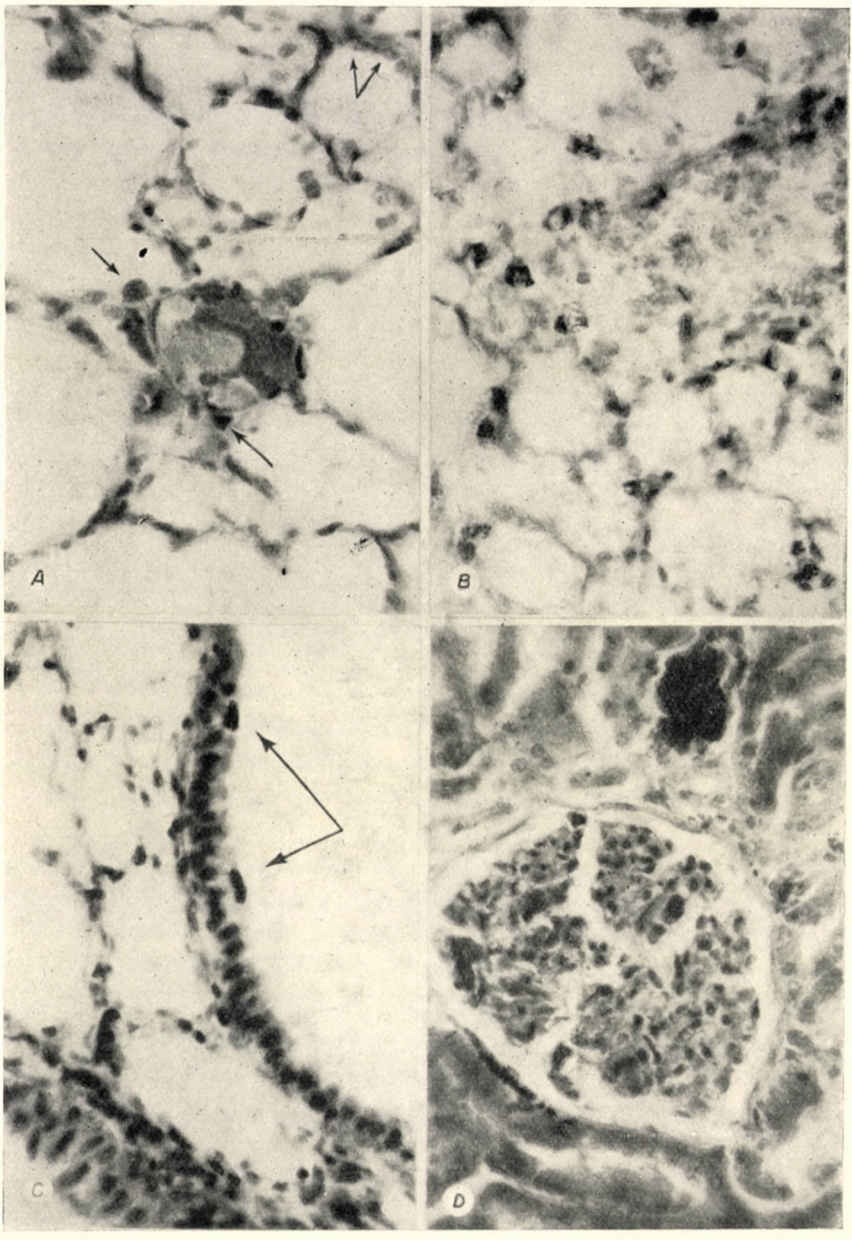

Fig. 2. Sections after intravenous injection

A. 1 to 2 minutes after injection : fine iron particles in macrophages above the vessels,
B. 10 minutes after injection : iron particles conglomerated in septal cells,
C. 5 minutes after injection : phagocytes loaded with particles on bronchiolar epithelium,
D. 72 hours after injection : particles in renal glomeruli and around Bowman's capsule. (In the mouse)

In the first 24 to 48 hours there were as yet a few particles in the regional lymph nodes, encountered mostly around the vessels.

Elimination by and storage in the kidney, liver, biliary tract, and the other organs, appeared negligible during the first 48 hours. The earliest to begin storage was the spleen where the particles were chiefly arranged extrafollicularly.

In 72 hours the macrophages passed through the walls of the smaller veins, and thereafter probably disintegrated since they could no longer be found in the systemic circulation. Around the veins phagocytes loaded with particles were encountered. After 4 to 5 days, permanent foci formed in the shape of small, reactive, perivascular nodules, in which the heavily loaded macrophages were surrounded by proliferating connective tissue cells.

These observations show the organism to possess an exceedingly swift and extensive disposition for mobilisation, and the lungs, which are not only the earliest but also the most potential storer of extraneous corpuscles, to play a decisive role in the process. This role of the lungs is but a transitory one since, counteracting the purposeful mechanism of storage, a minor part of the phagocytes returns the foreign matter to the circulation.

It was found that the mechanism at play in the case of an intratracheal introduction of the iron particles is more or less identical with that observed in connection with injections.

The coarser granules, though not in large amounts, invaded the lungs within the first 15 minutes, and very soon produced an irritation there which gave rise to cell proliferation and a fixing of the particles. Only a few of them passed from the lungs to the circulation, but only after some time, and if the dose of iron introduced through the trachea was large.

The fine granules, on the other hand, invaded the lungs in great masses within the first 15 minutes. They were seen temporarily in almost every alveolar cell (Fig. 3 A). At first they were scattered, but later clustered in some cells for transitional storage. Accumulation was again most marked in the perivascular and subpleural macrophages (Fig. 3 B). The alveolar walls taking up smaller quantities displayed early desquamation and an outward twist. Some perivascular macrophages were seen to pass through the vessel walls and break up in the circulation, but their number was much less than in the case of intravenous injection (Fig. 3 C). After the lapse of a few hours a fair amount of iron was found to accumulate temporarily in the septal cells.

After 72 hours very many perivascular and peribronchial foci were encountered consisting of cells capable of fixing particles.

Apparently, we have again failed in establishing whether or not endothelial cells possessed a phagocyting capacity. The majority of the particles reaching the lungs from without find their way into the alveolar cells, which, however, are cast off very soon. Some of the particles temporarily accumulate in the newly formed epithelial cells and interstitial macrophages. A further considerable part

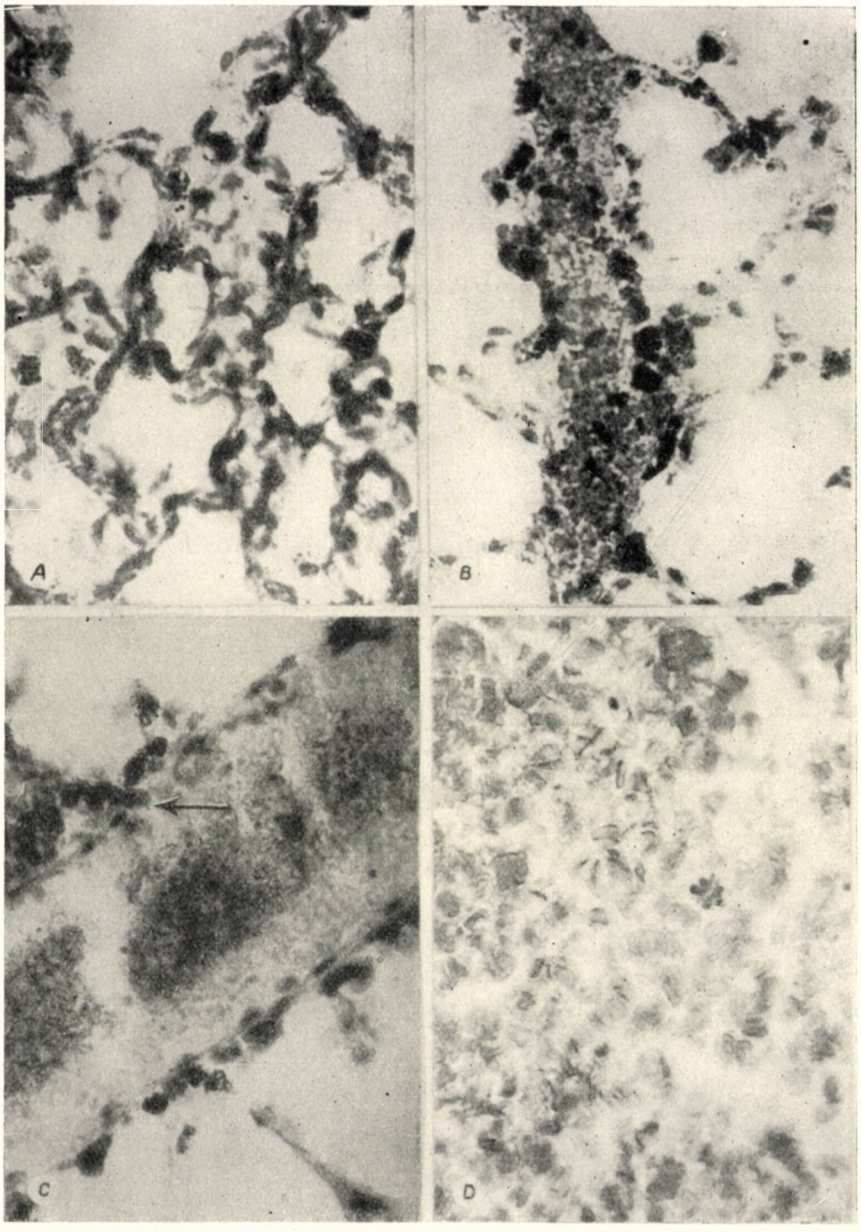

Fig. 3. Sections after intratracheal administration

A. 2 minutes after treatment : iron particles phagocyted by alveolar cells,
B. 5 minutes after treatment : particles conglomerated in pericytes in vessel lumen,
C. 10 minutes after treatment : numerous iron corpuscles in the middle of pulmonary vein, and macrophages penetrating through vessel wall,
D. 48 hours after treatment : free and phagocyted particles in the blood of the aorta

reaches the circulation without being phagocyted, and these, too, are first stored on the huge surface of the lung.

Summing up the differences between the storage of intravenously and intratracheally administered particles, the following can be said. Initially, most of the iron given intravenously is taken up by the septal cells, and most of that introduced intratracheally, by the alveolar cells.

Later, particles given intravenously are taken up by the alveolar cells, and intratracheally administered ones by the septal cells as well, for in this latter phase part of the corpuscles introduced through the trachea reaches the circulation and it is from there that the septal cells eliminate them.

Summary

Using the magnetic field and histochemical methods, the role of the lungs has been studied in storing and eliminating metal particles introduced into the animal organism intravenously and intratracheally, respectively. Particularly interesting are the observations made with particles from submicroscopic to 5 μ in diameter.

Not later than two minutes after the introduction of the "foreign" substance, the pulmonary cells take up the extraneous particles from the blood path and the alveoli. The lungs are the first among the organs not only to take up but also temporarily to store, by means of their septal and alveolar cells, the inorganic corpuscles introduced into the organism. Elimination of the "foreign" substance through the air passages begins through the intermediary of the pulmonary macrophages, but they appear to act as intermediaries in the opposite direction as well. This vitious circle is partly remedied by the reactive granulomata which form in a few days and store up the particles. At the beginning, storage, too, occurs chiefly in the lungs. Under the conditions of the experiments described the particles were being transported in the early phase through the blood vessels and not the lymphatics.

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РАННЯЯ РЕАКЦИЯ ОРГАНИЗМА НА ВНЕСЕНИЕ НЕОРГАНИЧЕСКИХ ЧАСТИЦ

И. ФОДОР и Г. МИШКОВИЧ

Авторам удалось магнитным и гистохимическим способами исследования сделать установления относительно накопляющей и выделяющей ролей легких путем внутривенного и интратрахеального введения металлических частиц, величиной от субмикроскопической до 5 микронов, в организм подопытных животных.

Клетки легких осваивают частицы железа из русла крови, и из альвеол уже по истечении двух минут после введения «инородного» вещества. Легкие не только усваивают, но и временно накопляют с помощью септальных и альвеолярных клеток внесенные в организм неорганические частицы. Деятельностью макрофагов легких начинается выделение «инородных» веществ через дыхательные пути, но кажется, что они осуществляют и посредничество противоположного направления. Порочный круг уменьшается образующимися в течение нескольких дней реактивными грануломами, которые накопляют частицы. Вначале накопление, главным образом, осуществляется также легкими. При данных экспериментальных условиях транспорт осуществляется в ранней стадии через кровеносные сосуды и не через лимфатические пути.

RÉACTION PRÉCOCE DE L'ORGANISME, APRÈS INTRODUCTION DE GRANULES ANORGANIQUES

I. FODOR et G. MISKOVITS

Nous avons pu faire des constatations concernant le rôle accumulateur et secréteur du poumon, en introduisant par la trachée et par voie intraveneuse des granules métalliques dans l'organisme d'animaux d'expérience. Nous nous sommes servis de méthodes histochimiques et magnétiques pour ces études. La grandeur des granules métalliques variait du submicroscopique à 5 microns.

Les cellules pulmonaires incorporent dès la deuxième minute après introduction de la substance «étrangère» les granules de fer, des vaisseaux sanguins et des alvéoles. De tous les organes, c'est le poumon qui le premier, non seulement prend en charge, mais aussi accumule provisoirement les granules anorganiques introduits dans l'organisme à l'aide de ses cellules septales et alvéolaires. C'est avec la participation des macrophages pulmonaires, que commence la secrétion des substances «étrangères» par les voies aériennes, mais il semble, qu'un processus intermédiaire en sens inverse ait lieu également. Le cercle vicieux est relenti par les granulomes réactifs, qui se forment en quelques jours et qui accumulent les granules. Au début, cette accumulation se fait surtout par le poumon. Dans les conditions expérimentales données, dans la phase précoce, le transport se fait par les vaisseaux sanguins et non par les vaisseaux lymphatiques.

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