

## STUDIES ON THE EFFECTS OF PHAGOCYTTIC STIMULATION ON MICROBIAL DISEASE

### XII. ACTION OF CHAULMOOGRA OIL ON THE RETICULOENDOTHELIAL SYSTEM<sup>1</sup>

LÁSZLÓ KÁTÓ, M.D.

AND BÉLA GÖZSY, D. PHARM., D. BIOCHEM.

*Institute of Microbiology and Hygiene, University of Montreal  
Montreal, Canada*

In the preceding paper of this series (4) it was reported that application of chaulmoogra oil and certain of its derivatives to the skin surface induced phagocytic activity of endothelial cells of the small skin vessels. The same phenomenon could be induced by histamine, as had previously been observed by Jancso (5) and confirmed by Biozzi *et al.* (1) and by ourselves (2). We attached a certain importance to this finding, because it revealed some preliminary evidence of the mechanism of action of chaulmoogra derivatives on the host. It appears that under the influence of these substances the endothelial cells undergo a functional metamorphosis. Normally, they have no phagocytic activity, but they may acquire it under the influence of histamine or chaulmoogra derivatives. The influence of either substance, histamine or chaulmoogra derivative, was inhibited by the previous administration of synthetic antihistaminic substances.

Jancso also demonstrated that if albino mice or rats were injected with an antihistaminic substance, india ink injected intravenously disappeared from the blood stream of these animals more slowly than was the case with control animals or with histamine-treated animals. We have reported (6) that histamine, and certain substances thought to be histamine liberators, stimulate the reticuloendothelial system (RES) to increased phagocytic activity (3). Once it was seen that the effect of chaulmoogra oil on the endothelial phagocytosis was similar to that of histamine, it became necessary to investigate experimentally whether chaulmoogra oil would similarly stimulate the phagocytic activity of the RES.

#### MATERIAL AND METHODS

Experiments were conducted as described by Jancso, using 250 albino rats weighing 150-175 gm. each, in four groups. They were fed in the morning and injected three hours later. Group 1, 50 animals, received subcutaneously histamine bichlorhydrate in a saline solution, 2 mgm./100 gm. body weight. Group 2, 50 rats, was injected subcutaneously with 8 mgm. of diphenhydramine hydrochloride (Benadryl) in saline solution. Twenty-four hours, and again 1 hour, before the administration of india ink, Group 3, also 50 rats, received 0.5 cc. of chaulmoogra oil intramuscularly.

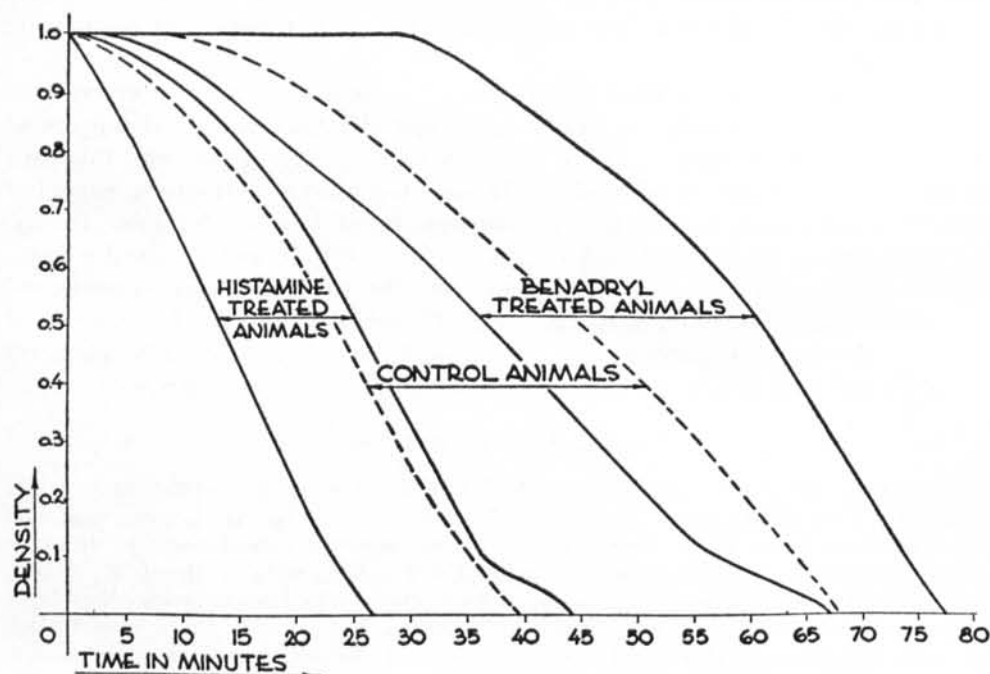
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One hundred animals served as a control group and received the solvents. In the first two groups, 30 minutes after the earlier injection, 1.5 cc. of 15 per cent Pelikan india ink containing 1 per cent gelatin and 0.8 per cent sodium chloride in aqueous solution was injected intravenously into the tail vein of each animal. In Group 3, the same india ink injections were made 1 hour after the second chaulmoogra oil injection. The animals of the control group received the same amount of india ink.

Every fifth minute after the injection of the ink, blood was taken from the tail vein of each animal of every group and dropped on a strip of fine filter paper (Talquist hemaglobin paper). Only two rats were worked on at one time, by a skilled person. The average diameter of the blood spot was 1 cm. Blood samples were taken in this way until the india ink particles completely disappeared from the blood stream. The blood spots were allowed to dry and the hemoglobin was dissolved from the paper in a bath of 1 per cent sodium carbonate. There remained on the paper slides a series of blackish to grayish spots, illustrating the progressive diminution of india ink concentration in the blood stream. The optical density of each blood spot as compared with that of the filter paper itself was measured by transmitted light by means of a photoelectric cell. By this method the relative quantities of circulating india ink were known by density and by elapsed time, and the variations in density were plotted against time in minutes on scale paper.

#### RESULTS

The differences in the rapidity of disappearance of the india ink particles from the blood stream of the 50 antihistamine-treated animals and the 50 histamine-treated animals, as compared with the control group of 100 animals are shown in Text-figure 1.



TEXT-FIG. 1. Graph showing the rapidity of disappearance of india ink from the blood stream of rats treated with histamine and with the antihistaminic diphenhydramine hydrochloride (Benadryl), as compared with the control group.

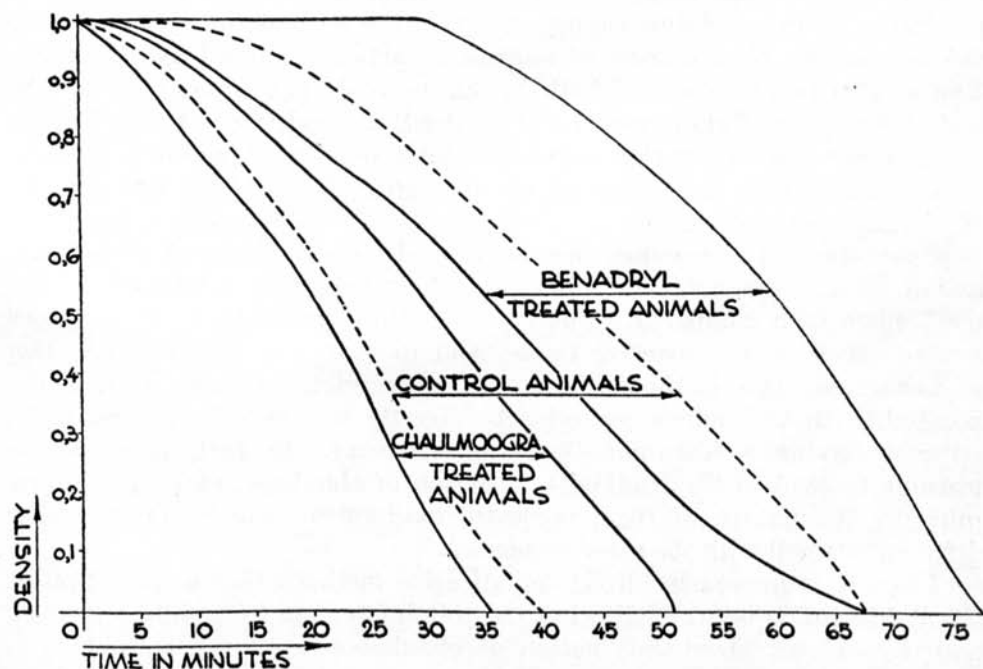
The differences between the rapidity of disappearance of india ink from the blood stream of 50 chaulmoogra oil-injected animals and the same phenomenon in the control and antihistamine-injected groups are shown in Text-figure 2.

As shown in the first graph, the carbon particles were completely removed from the circulating blood by the RES of the control animals in from 39 to 66 minutes, while the RES of the antihistamine-treated rats took from 67 to 77 minutes to perform the same function. From an examination of both graphs it will be seen that a period of only 27-44 minutes was needed by the histamine-treated group, and one of 36-51 minutes by the chaulmoogra-treated group, for the complete removal of the ink. These results demonstrate that both histamine and chaulmoogra oil increase the rapidity of disappearance of india ink particles from the blood stream.

*Combined experiment.*—In another group of 50 rats injected simultaneously with both chaulmoogra oil and antihistamine, in the same amounts as before, the india ink was removed by the RES with the same rapidity as in the control group of animals. The two substances evidently neutralized each other.

#### DISCUSSION

Halpern *et al.* (7) injected india ink intravenously into mice and rats and determined, for specified periods, the amount of ink circulating in



TEXT-FIG. 2. Graph showing the rapidity of disappearance of india ink from the blood stream of rats treated with histamine and with the antihistaminic diphenhydramine (Benadryl), as compared with the control group.

the blood stream. If 8 or 16 mgm./100 gm. body weight was injected, they found that the ink particles were ingested only by the cells of the RES, mainly in the lungs, liver and spleen, while only small amounts were accounted for by the kidneys and bone-marrow.

From our experiment we have information on the phagocytic function of the RES under the influence of various introduced substances, and since the proportion of injected india ink is substantially the same the experimental conditions may be compared with those of Halpern's experiment. Since he found that about 95 per cent of the injected ink was taken up by the RES cells of the spleen, liver and lungs, we may assume that if the rapidity of storage of particles is increased or decreased because of an introduced substance that substance acts directly on the cells of the RES, stimulating or slowing their phagocytic activity. On this basis we may conclude from our observations that both histamine and chaulmoogra oil stimulate the RES to increased phagocytic activity, whereas antihistamine slows down that activity.

In our preceding paper (4) we showed that both histamine and chaulmoogra oil induce phagocytic activity of the endothelial cells of skin capillaries, and that synthetic antihistamines inhibit this phenomenon. In the present experiment, there is a surprising similarity in the effects of histamine and chaulmoogra oil on the RES. The antihistamine, on the other hand, slowed the normal rate of phagocytosis, and when given together with chaulmoogra oil it neutralized the stimulating effect of the latter. Previously (6), we reported that the antihistamine antagonized the accelerated phagocytosis of india ink particles induced by histamine. The similarity of action of both histamine and chaulmoogra oil on the endothelial cells of skin vessels and on the RES, and the fact that in both experiments the stimulation could be inhibited by antihistamine, permits us to assume that the action of chaulmoogra oil may be a consequence of histamine liberation.

Synthetic antihistamines are of very different chemical structures, and it seems reasonable to think that their action is not based on the mechanism of a competitive antagonism. More probably, antihistamines are adsorbed on the reactive tissue, and in that way inhibit the action of histamine. If this theory is correct we cannot, however, exclude the possibility that chaulmoogra oil acts directly on the cells of the RES, without having a histamine-liberating capacity. In fact, it might be possible to explain the similarity of action of chaulmoogra oil and histamine by the nature of the phagocytic mechanism, which may react to different stimuli with the same response.

Until it is proved by direct quantitative methods that a liberation of latent histamine occurs following the administration of chaulmoogra oil, both hypotheses about this action of chaulmoogra oil are possible.

#### SUMMARY

Four groups of albino rats were injected intravenously with india ink after the parenteral administration of various substances. In the

control animals, the ink disappeared from the blood stream in 39 to 68 minutes. The administration of synthetic antihistamine slowed the rapidity of the phagocytosis of the ink particles by the reticuloendothelial system, whereas the administration of histamine, and also of chaulmoogra oil, increased the rapidity of phagocytosis. The increased phagocytic activity thus induced by chaulmoogra oil was found to be inhibited by the simultaneous administration of an antihistamine substance. These experiments have brought further evidence about the mechanism of action of chaulmoogra oil in the treatment of leprosy, namely, that it stimulates the cellular defense mechanism of the host to increased activity.

#### RESUMEN

A cuatro grupos de ratas albinas se les inyectó intravenosamente tinta china después de la administración parentérica de varias substancias. En los animales testigos, la tinta desapareció del torrente sanguíneo en 39 a 68 minutos. La administración de antihistamina sintética retardó la rapidez de la fagocitosis de las partículas de tinta por el sistema reticuloendotelial, en tanto que la administración de histamina, y también de aceite de chaulmugra, acrecentó la rapidez de la fagocitosis. La mayor actividad fagocítica provocada en esa forma por el aceite de chaulmugra se mostró inhibida por la administración simultánea de una substancia antihistamínica. Estos experimentos han aportado nuevos datos acerca del mecanismo de acción del aceite de chaulmugra en el tratamiento de la lepra, a saber, que excita a mayor actividad el mecanismo de defensa celular del huésped.

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