ACTION OF HISTAMINE AND ANTIHISTAMINE ON THE INGESTION OF MURINE LEPROSY BACILLI BY MACROPHAGES OF THE RAT AND THE GUINEA-PIG.[1]

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The discovery of synthetic antihistaminic substances has had a great influence on studies of the cellular defense mechanism. Experiments with antihistamines have permitted better understanding of the functioning of certain defence processes. These substances inhibit the formation of the first barrier against bacterial invaders, and also the phagocytic activity of fixed (8, 9, 10) and mobile (4, 10, 11) cells of the reticuloendothelial system. They also inhibit the action of certain mediators which maintain important functions of the defence apparatus (12, 13). Consequently, they aggravate acute and chronic bacterial infections (3, 5, 6).

We have found (3) that synthetic antihistamines also have a deteriorating effect in experimental tuberculosis in guinea-pigs and mice. Later, we showed that a synthetic antihistamine (mepyramine maleate) had a restraining effect on the phagocytosis of tubercle bacilli by monocytes (10); and that, contrarily, histamine stimulated the monocytes to an increased phagocytic activity toward the same bacilli (4).

We then turned our attention to murine leprosy, another disease in which the mononuclear macrophages play an important role. The purpose of the present investigation was to ascertain whether a similar mechanism could be detected in the macrophage-parasite relationship in murine leprosy. The first observation was contrary to that which was found with tubercle bacilli: the antihistamine did not inhibit the phagocytosis of *Mycobacterium leprae murium* by monocytes of guinea-pigs. This negative result stimulated the experiments which are reported here, designed to determine the influence of histamine and antihistamine on the phagocytosis of BCG and *M. leprae murium* by the monocytes of guinea-pigs and Wiersing rats.

MATERIALS AND METHODS

Animals.—Guinea-pigs of both sexes, weighing 450-500 gm., were used. The Wiersing rats used weighed 80-90 gm. The animals were fed with pellet diet, the guinea-pigs receiving also cabbage *ad libitum*.

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Substances used.—The affective substances employed were histamine bichlorhydrate, and the synthetic antihistamine mepyramine maleate (Neo-Antergan), dissolved in Hanks' balanced salt solution.

Macrophage suspension.—Guinea-pigs were injected intraperitoneally with 10 cc. of saline containing 0.1 mgm. of glycogen, and five days later they were killed by bleeding from the heart. The peritoneal cavity was washed out with 30 cc. of Hanks' solution containing 1:40,000 heparin, and the exudate, containing mainly mononuclear phagocytes, was diluted to contain 1,500-2,000 cells per cu.mm. and kept in a water bath at 37°C. Fresh guinea-pig serum, 2.5 per cent, was added to the suspension.

Exudates from the Wiersing rats were obtained and prepared in the same way, except that the rats were injected with 3 cc. of the glycogen-saline solution, and they were killed nine days later.

Preparation of tubercle bacilli (BCG.)—The BCG strain used was cultivated for 14 days in Dubos' Tween-albumin liquid medium, and the bacilli were washed three times with saline and a fourth time with Hanks' solution. The final centrifuging was for 3 minutes at 220G., in order to obtain a clump-free suspension. A 1:10 dilution of the culture was used for the parasitization.

Preparation of rat leprosy bacilli.—M. leprae murium, of a strain kindly supplied by Dr. E. Grunberg, was maintained in Wiersing rats by transfer at 12-week intervals, as described by Grunberg and Schnitzer (2). A 12-weeks-old leproma, non-necrotic and free of gross connective tissue, was ground with sand and diluted to 1:10 with saline solution. The suspension was passed through rapid filter paper and centrifuged for 3 minutes at 200G. It was then washed once with saline, and suspended in Hanks' solution. This crude preparation, which contained some tissue fragments, mainly destroyed macrophages, was diluted to contain approximately 30,000-40,000 bacilli per cu.mm., counting against red blood cells.

Parasitization of macrophages.—To 2 cc. of the macrophage suspension the desired amount of histamine or antihistamine was added in 0.25 cc. solution, and, after 10 minutes in a water bath at 37°C, 0.25 cc. of the bacillus suspension was added. The phagocyte mixture was then poured over a previously prepared coverglass in a small plastic dish (23 mm. in diameter and 4 mm. high). This preparation was incubated at 37.5°C for 60 minutes, during which time the cells settled on the coverglass. Readings were made by examination with the Leitz contrast phase microscope, and examinations for possible contamination were made by Aubert's cold-staining method (1). One hundred monocytes were counted on each slide, and the percentages of cells that contained 0, 1-2, 3-5, 6-10, and more than 10 bacilli were recorded.

All manipulations were performed under as nearly aseptic conditions as possible, and no penicillin was used. Every preparation was made in duplicate. Contaminated samples were discarded.

RESULTS

With the cells of the nonsusceptible animal, the guinea-pig, histamine in 10 μgm./cc. concentration increased the phagocytic activity against BCG, as is to be seen from the first section of Table 1 in comparison with the control.[2] Monocytes of control preparations, and in the presence of less than 10 μgm./cc. histamine, presented huge pseudopodia and large vacuoles in the cytoplasm (Fig. 1). On the other hand it will be seen that the antihistamine, even in very small amounts, reduced considerably

[2] It may be noted that in previous work (4) it was found that if the concentration of histamine was increased to 100 μgm./cc. the phagocytic activity was progressively lowered.

the phagocytosis of BCG. Even in the presence of histamine, the antihistamine in sufficient amount reduced markedly the phagocytic activity of the cells.

Regarding the condition of the monocytes, those treated with histamine showed good ameboid movement, whereas if the antihistamine

TABLE 1.—*Phagocytosis of BCG by (a) guinea-pig monocytes and (b) rat monocytes as influenced by histamine and the antihistaminic substance (mepyramine maleate).*

Substance added and amount (μgm./cc.)		Number of bacilli per monocyte[a]				
		0	1-2	3-5	6-10	>10
(a) Guinea-pig monocytes						
None (control)		25	30	31	12	2
Histamine	10.00	13	24	33	17	13
Antihistamine	0.05	48	34	15	2	1
	0.10	48	29	14	6	3
	0.50	55	25	16	4	0
	1.00	88	5	4	3	0
	10.00	86	10	2	1	1
Histamine	10.00					
Antihistamine	0.10	14	26	30	23	7
	1.00	15	15	31	27	12
	10.00	90	7	3	0	0
(b) Wiersing rat monocytes						
None (control)		34	36	27	2	1
Histamine	10.00	11	17	38	16	18
Antihistamine	0.10	31	34	28	5	2
	1.00	51	26	23	0	0
	10.00	58	31	10	1	0
	100.00	82	13	5	0	0

a Percentages of counts of 100 cells each.

was present in more than 1 μgm./cc. concentration the cells were much affected. They were rounded, smaller than the controls, and nonmotile; they showed no pseudopodia and only a few showed vacuoles, which were very small; and they stained dark or absorbed transmitted light to a large extent (Fig. 5).

The findings with BCG and the monocyte from the susceptible animal, the rat, were very similar as shown in the second section of Table 1. The

cells without the test substances were similarly active (Fig. 2). The only difference was that the antihistamine reduced the phagocytosis to a lesser extent than with the monocytes of the guinea-pigs.

Next we investigated the behavior of macrophages from both animals with regard to the described suspension of *M. leprae murium*. With the guinea-pig macrophages these bacilli in control preparations were rapidly ingested, as shown in the first section of Table 2. Histamine failed to stimulate this phagocytosis further, although it did so with BCG in the earlier experiment. Furthermore, the antihistamine—again in contrast to the effect with BCG—did not in this case inhibit the phagocytic activity.

TABLE 2.—*Phagocytosis of murine leprosy bacilli by macrophages of the guinea-pig, without and with histamine and antihistamine (mepyramine maleate); (a) with the original bacillus suspension, and (b) with the thrice-washed suspension.*

Substance added and amount (μgm./cc.)		Number of bacilli per monocyte[a]				
		0	1-2	3-5	6-10	>10
(a) Original bacillus suspension						
None (control)		6	9	28	28	29
Histamine	25	12	16	27	26	19
Antihistamine	10	8	27	23	22	20
	25	4	26	36	30	4
(b) Thrice-washed bacillus suspension						
None (control)		28	53	12	6	1
Histamine	10	11	50	27	8	4
	100	9	52	25	12	2
Antihistamine	10	70	24	5	1	0
	100	84	12	3	1	

a Percentages of counts of 100 cells each.

An important observation was made with respect to the morphology of the phagocytes. Those in the control preparation (Fig. 3) showed no pseudopodia, or only small ones, although they ingested large numbers of the murine bacilli. The cells were of shrunken appearance, small, rounded and dark, very similar to those in the BCG experiment under the influence of the antihistamine (Fig. 5). We gained the impression that the paralyzed cells were actively invaded by the bacilli, and that the behavior of the cells themselves was passive.

The findings with the monocytes derived from the rat were similar. Again their phagocytic activity was not inhibited by the antihistamine, and the morphological peculiarities were similar (Fig. 4).

Also of interest is the fact that the murine bacilli were ingested by the phagocytes in greater numbers than were the tubercle bacilli. This difference could not be attributed to the fact that the murine bacillus suspension contained more microorganisms than the BCG suspension. Since in this case the antihistamine had failed to inhibit the phagocytosis of the murine leprosy bacilli, it seemed possible that the tissue debris of the incompletely-washed suspension might be responsible for this finding.

To investigate this possibility the murine bacillus suspension was washed again twice in the Hanks' solution to eliminate the tissue fragments. The results of comparisons of the two suspensions are shown in Table 3. The extra treatment reduced materially the numbers of bacilli taken up.

TABLE 3.—*Phagocytosis of once- and thrice-washed murine leprosy bacilli by the guinea-pig macrophage.*

Leproma suspension	Number of bacilli per monocyte[a]				
	0	1-2	3-5	6-10	> 10
First experiment					
Once-washed	7	19	41	25	8
Thrice-washed	22	33	34	8	3
Second experiment					
Once-washed	5	14	43	20	18
Thrice-washed	22	18	24	26	10

a Percentages of counts of 100 cells each.

Before we could proceed with tests with histamine and the antihistamine the thrice-washed suspension was kept at 4°C overnight. It will be seen from the second section of Table 2 that with the thrice-washed bacillus suspension those substances now had the same effect they had with the BCG-culture suspension, i. e., stimulation by the histamine and inhibition by the antihistamine.

From this result it seemed obvious that one or more substances were present in the crude suspension of the murine leproma, presumably derived from the leprous tissue itself, which inhibits the action of histamine and the antihistamine on the murine bacilli. We, therefore, proceeded to prepare a bacillus-free leproma extract and ascertain its effect on the phagocytosis of the tubercle bacilli by guinea-pig cells. The ground-up leproma, suspended 1:10 in saline, was subjected to prolonged centrifuging until the supernatant contained only a very few acid-fast frag-

ments. This raw leproma extract proved to be highly inhibitory of the phagocytosis of BCG by the guinea-pig monocytes, as shown in Table 4. This inhibition was not influenced by added antihistamine, and was only slightly reduced by 10 μgm./cc. of histamine.

TABLE 4.—*Ingestion of BCG by guinea-pig monocytes as influenced by the rat leproma extract, and by histamine and antihistamine (mepyramine maleate).*

Substance added and amount (μgm./cc.)	Number of bacilli per monocyte[a]				
	0	1-2	3-5	6-10	> 10
None (controls)	28	29	26	12	5
	32	20	27	10	11
Leproma extract only	92	8	0	0	0
Leproma extract plus Histamine 10	66	18	14	2	0
100	38	24	14	12	12
Leproma extract plus Antihistamine: 1	94	6	0	0	0
10	79	14	6	1	0

a Percentages of counts of 100 cells each.

The phagocytic monocytes showed a marked morphological change under the influence of the leproma extract. They showed no movement, and most of them had no pseudopodia or vacuoles; and they became smaller than those in the control, the unusual shrinkage causing them to assume the appearance of lymphocytes (Fig. 6).

An experiment was carried out to ascertain the effect of dilution of the fresh leproma extract. As shown in Table 5, a 10^{-3} dilution still

TABLE 5.—*Phagocytosis of BCG by guinea-pig monocytes as influenced by the rat leproma extract.*

Rat leproma extract added	Number of bacilli per monocyte[a]				
	0	1-2	3-5	6-10	> 10
None (control)	18	32	34	9	7
Dilution 10^{-1}	77	16	7	—	—
Dilution 10^{-2}	71	12	12	3	2
Dilution 10^{-3}	54	25	16	4	1

a Percentages of counts of 100 cells each.

induced a noticeable reduction in the number of tubercle bacilli ingested by the monocytes.

These preliminary experiments suggested that there is present in the rat leproma a highly active substance, one which surely must play an important role in the pathogenesis of the disease. Further study has been aimed at the purification and identification of this factor, and the role of the substance in experimental murine leprosy is under investigation.

DISCUSSION

Experiments with surviving monocytes may be productive of important information about the macrophage-parasite relationship in experimental rat leprosy. The present investigation concerns mainly the influence of histamine and an antihistamine on the ingestion of the rat leprosy bacillus by the mononuclear cells of peritoneal exudates. A basic difference has been found between that phenomenon and the ingestion of tubercle bacilli (BCG). Phagocytosis of BCG by the monocytes of both the guinea-pig and the rat is stimulated by histamine and inhibited by the synthetic antishistamine, whereas these substances did not have those effects, with the macrophages of either species, on ingestion of rat leprosy bacilli of the crude suspension originally prepared.

A factor, doubtless complex, can easily be extracted from the rat leproma which inhibits the phagocytosis of BCG by the guinea-pig monocytes, an effect which is not overcome by histamine. This extract is very active, for a 10^{-3} dilution was still found to be effective.

Although the substance prevented the ingestion of the tubercle bacillus by the guinea-pig monocytes, which assumed a passive behavior in its presence, when the murine bacillus was used the situation was quite different. Large numbers of the bacilli invaded the shrunken, quite immobile monocytes, and neither histamine nor the antihistamine had any effect. However, when the murine-bacillus suspension was freed of the unknown factor by washing, the bacilli were ingested in smaller numbers, and then phagocytosis was stimulated by histamine and inhibited by the antihistamine, as with the tubercle bacillus.

On the basis of these observations we are tempted to say that ingestion of murine leprosy bacilli by the monocytes cannot be considered a real phagocytosis, since these bacilli invade the monocytes in large numbers although the cells are paralyzed by a factor present in the leproma extract. This paralyzing action of the extract on the cells is not identical with the similar action of the antihistamine, since the extract permits not only the ingestion of large numbers of the bacilli, but also an undisturbed symbiosis of the macrophage and the parasite.

Morphologically, the macrophages have similar characteristics under the influence of the antihistamine and the leproma extract (Figs. 5 and 6). Both substances prevent phagocytosis of BCG and cause shrinkage of the cells, characterized by their small, round form and absence of

pseudopodia. The origin and nature of the factor in the leproma extract is not evident. Whether it is a metabolic product of *M. leprae murium* or of the parasitized macrophage, or is a result of tissue destruction in the granuloma, its interesting property (responsibility for the monopolization of phagocytic cells by the murine bacillus) merits attention.

The observations also gave further evidence of the difference as regards the host-cell response to the parasite in experimental tuberculosis and experimental rat leprosy. Monocytes of both the susceptible and the resistant animals were rapidly invaded by the murine bacilli, which were thus quickly removed from the unfavorable extracellular environment (7). With this bacillus the antihistamine could not prevent the ingestion phase of the phagocytic cell function, whereas it did so with the tubercle bacillus. These observations must be interpreted very carefully, mainly because the material derived from the rat leproma contains a series of unknown factors. As a chronic granulomatous process the rat leproma contains substances deriving from tissue destruction. Whether these substances have a decisive effect on the outcome of the disease could be postulated from the experiment of Menkin (12) and Spector (13). How far the presence of Menkin factors influenced the phenomena observed cannot be concluded from the experiments, but the observations suggest that phagocytosis of murine bacillus is dependent on a very different mechanism from that of the tubercle bacillus.

SUMMARY

Phagocytosis of the tubercle bacillus of a BCG culture, and of the murine leprosy bacillus of a leproma suspension, by macrophages of glycogen-induced peritoneal exudates of guinea-pigs and albino rats was studied to determine the influence of histamine and an antihistamine (mepyramine maleate). Ingestion of BCG by the cells of both species was stimulated by histamine and inhibited by the antihistamine. On the other hand, phagocytosis of the murine bacillus was not influenced by either of the two substances, this contrary result being obtained with both kinds of exudate cells. A raw, bacillus-free, aqueous extract of the rat leproma inhibited phagocytosis of BCG by the guinea-pig monocytes. The active substance is considered responsible for the monopolization of the monocytes by *M. leprae murium*. The rat leproma extract and the antihistamine induce similar morphological and functional alterations of the monocytes; absence of pseudopodia and vacuoles, shrinkage of the cell, and a passive behavior with regard to BCG.

RESUMEN

A fin de determinar el influjo de la histamina y de una antihistamina (maleato de mepiramina), se estudió la fagocitosis del bacilo tuberculoso de un cultivo del BCG y la del bacilo leproso murino de una suspensión de leproma, por macrófagos de exudados peritoneales de cobayos y ratas albinas, inducidos por glucógeno. La ingestión del BCG por las células de ambas especies fué excitada por la histamina e inhibida

por la antihistamina. En cambio, la fagocitosis del bacilo murino no fué afectada por ninguna de las dos substancias, obteniéndose este efecto contrario con ambas clases de células del exudado. Un extracto acuoso crudo, privado de bacilos, del leproma de rata inhibió la fagocitosis del BCG por los monocitos de cobayo. Se considera que la substancia activa es la responsable de la monopolización de los monocitos por el *M. leprae murium*. El extracto de leproma de rata y la antihistamina inducen semejantes alteraciones morfológicas y funcionales de los monocitos: falta de pseudópodos y vacuolas, contracción de la célula y comportamiento pasivo con respecto al BCG.

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Description of Plate

Plate 13

All photomicrographs were made with the Leitz contrast-phase microscope and the Leica camera, using Anscochrome tungsten-type film.

Fig. 1. Phagocytosis of BCG by macrophages of the guinea-pig. Note the large pseudopodia and vacuoles in the cytoplasm.

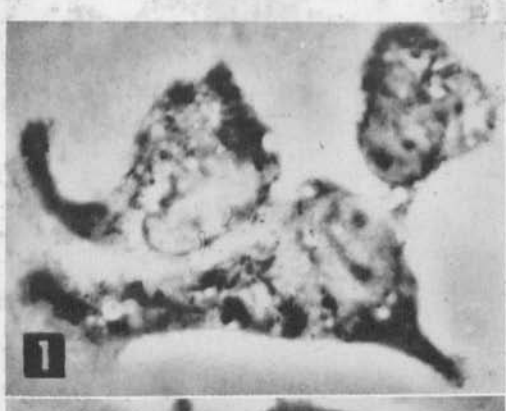
Fig. 2. Phagocytosis of BCG by macrophages of the albino rat. Note the pesudopodia and vacuoles. These cells were as active as were those of the guinea-pig (Fig. 1).

Fig. 3. Phagocytosis of rat leprosy bacilli (original suspension) by macrophages of the guinea-pig. These cells showed few and only small pseudopodia and were inactive, but nevertheless contained large numbers of the bacilli.

Fig. 4. Phagocytosis of rat leprosy bacilli by macrophages of the rat. In general these cells were very similar to those of the guinea-pig under the same conditions (Fig. 3).

Fig. 5. Phagocytosis of BCG by macrophages of the guinea-pig in the presence of 10 μgm./cc. of mepyramine maleate. The cells are smaller, more rounded than in the controls (Fig. 1), and nonmotile. The tubercle bacilli are found mainly extracellularly. (The original picture, before cropping, showed free-lying bacilli much as in Fig. 6.)

Fig. 6. Phagocytosis of BCG by macrophages of the guinea-pig as influenced by the bacillus-free rat leproma extract. Note the dense, rounded form of the macrophages and the extracellular tubercle bacilli.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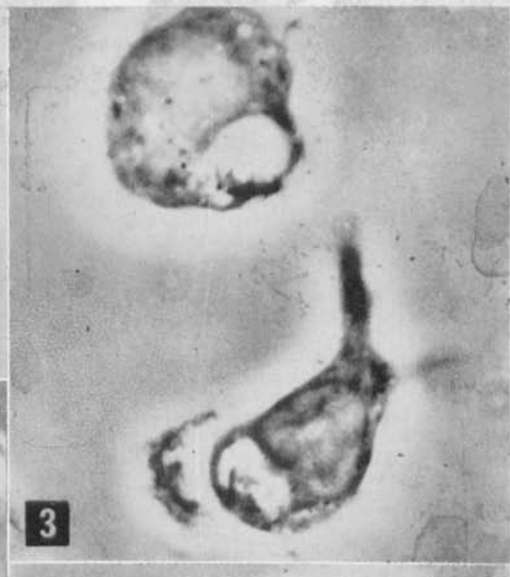
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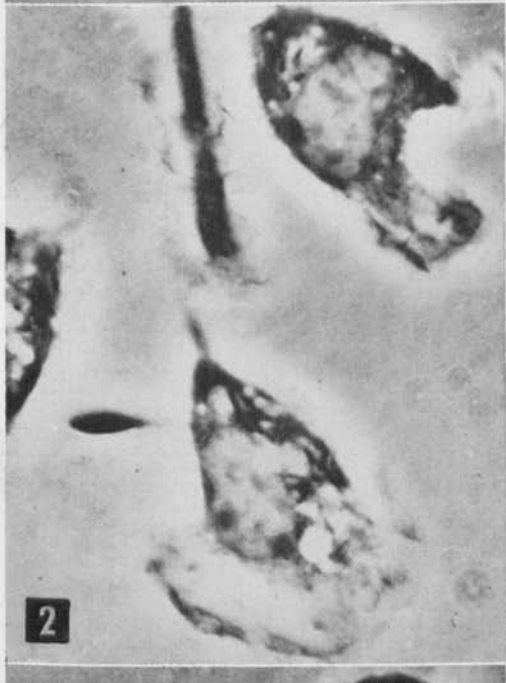
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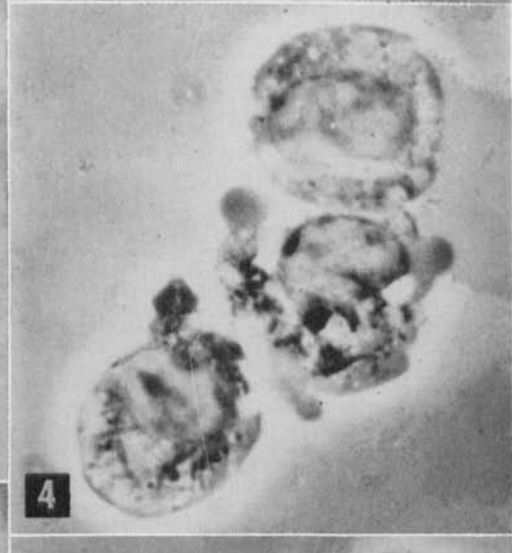
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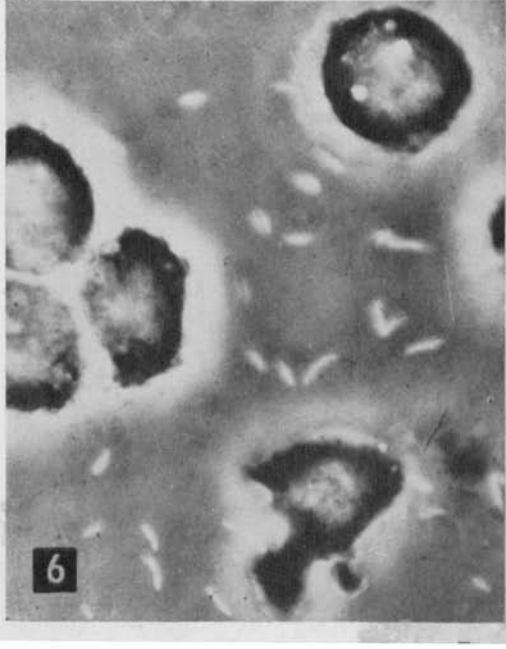
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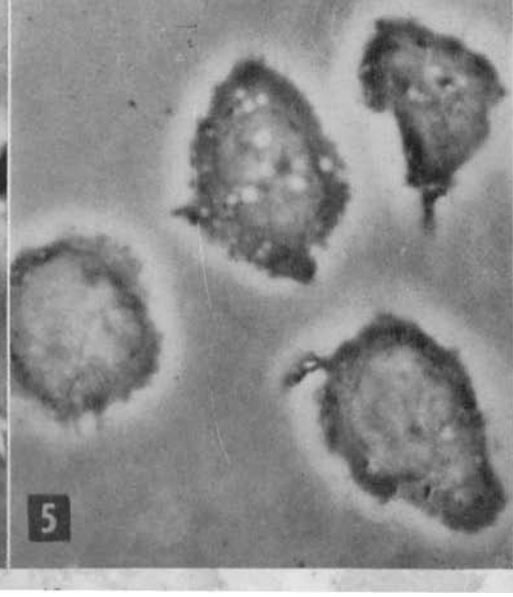
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