nonobese animals, given the opportunity to exercise by being transferred to activity cages, lose some weight during the first 2 weeks (weight then stabilizes and activity rates decrease slightly), no such reaction is demonstrated by the young or the older obese animals. In much older animals not included in the main experiment (age over 8 months, weight over 60 g) the number of revolutions/day dropped to less than 2 or 3.

The fact that decrease in activity precedes marked obesity makes it possible to ascribe to this decrease in activity a role in the etiology of the obesity. The weight differences between obese and nonobese animals have been found to be accounted for almost exclusively by fat (*11*). Weight increases of 15–20 g/month in adult obese mice are frequently observed. The young obese mice in the experiment reported here, for example, were accumulating fat at the rate of 16 g/month. It is therefore readily seen that differences of food consumption between obese and nonobese animals of the order of 25% (*1*) can account for the development of the obesity only if the extra 5 calories/day consumed by the obese are converted into body fat with a net efficiency of about 100% instead of one of the order of 25%, as previously postulated. In some cases the increase in food intake over the nonobese level cannot *per se* account for the obesity. The explanation for this fact, as well as for the improbable efficiency level, lies in two findings: (*a*) The resting metabolism stays the same when the body weight increases by 200 or 300% (*5*); in fact, the total oxygen consumption per animal is somewhat lower in obese than in nonobese animals. (*b*) Far less energy is used in movement by the obese than by the nonobese animals, and the cost of moving the body weight does not increase as obesity develops because activity is almost nil. It can be calculated from respiratory data (*5*) that nonobese animals require 11 cal/day for basal expenditure. If specific dynamic action is taken as 10% of the total intake of 20 cal, it is readily seen that 7 cal are left for spontaneous activity and some fat formation. The same calculations applied to obese animals give 9.5 cal for basal expenditure, 2.5 for specific dynamic action, and 13 cal for fat formation and exercise, with the latter a negligible item. Decrease in activity therefore represents a not unimportant factor in the etiology of this form of obesity.

Two other observations are relevant to the problem of the relation of exercise to the development of obesity in these mice. First, when obese mice carry the waltzing gene and are in constant rotary movement in their cages, their weight rarely exceeds 40 g instead of twice that value. Second, it has been shown previously (*7*) that when obese mice are pair-fed with nonobese litter mates, their weight stabilizes at the level reached before paired feeding was started, neither increasing nor decreasing. As the resting over-all metabolism of obese animals is not greater than that of nonobese, they should, if their expenditure for work remained lower than that of the nonobese, still gain weight under paired feeding conditions. Actually, only a few mice do. The explanation of this apparent paradox lies in the fact that total or partial fasting increases the activity of obese mice proportionally much more than that of the nonobese. Total deprivation of food increases the number of revolutions per day by 50–60% for the nonobese—it brings it up to normal nonfasted levels (4000–5000) for young obese animals. Paired feeding, which represents a curtailment of intake of about 25% for the obese, is found to bring about a rate of activity of 1000–2000 revolutions per day. When the heavier weight of the obese animals is taken into account, the increase in work expenditure added to the decrease in caloric intake is seen to be sufficient to account for the cessation of weight increase.[3]

The relation of the decreased activity of the obese animals to their decreased resistance to cold is discussed in another publication (*12*), as is the relation of activity to human obesity (*13*).

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[3] In private conversation with Thomas H. Maren, of the American Cyanamid Co., it was found that he had independently arrived at the conclusion (unpublished) that part of the excess fat of the obese animals must be derived from energy devoted to exercise in the nonobese animals.

## Mast Cells and Susceptibility to Experimental Atherosclerosis[1]


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It has been known for the past four decades that lipemia and atherosclerosis can be produced easily by cholesterol feeding in the rabbit, whereas the rat is a notoriously refractory species (*1*). The reason for the remarkable resistance of the latter species is unknown and has been the subject of considerable speculation. It has been attributed in turn to a very efficient vasa vasorum system, to a very low resting blood cholesterol

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level, and to an unusually rapid clearance of intravenously injected cholesterol (*1*).

In recent years, heparin has been shown to prevent lipemia following fat meals (*2*) and to "wipe out" the giant lipoprotein molecules that appear in the blood of atherosclerotic patients and cholesterol-fed rabbits (*3*). Furthermore, in agreement with the claim of Graham *et al.* (*4*), we have presented evidence that heparin will retard the development of atherosclerosis in cholesterol oil–fed rabbits (*5, 6*).

In view of these data, and since it is highly probable that heparin is produced by the mast cells in the connective tissue (*7, 8*), we compared the histological mast cell content of numerous organs from the rat and the rabbit.

The following materials were examined in each of 4 Wistar albino rats (3 males and 1 female): skin, skeletal muscle, heart, brain, aorta, thymus, lung, liver, spleen, kidney, pancreas, jejunum, mesentery, and omentum. The same materials and, in addition, the adrenal, the gonad, and the uterus or epididymis were examined in each of 5 white rabbits (3 females and 2 males).

All organs were fixed in a mixture of 16 parts 95% alcohol, 2 parts 40% formaldehyde, and 1 part glacial acetic acid. In all instances, random sections from the whole organ were stained as follows: (1) aqueous 0.5% toluidine blue, slightly alkaline; (2) aqueous 0.5% thionine blue, slightly alkaline; (3) aqueous 0.5% toluidine blue, buffered at pH 4.5–4.7 (*9*); (4) alcoholic 0.5% thionine blue (in 70% alcohol) with no solution of less than 70% alcohol concentration used between deparaffinization and mounting (von Möllendorf's all-alcoholic procedure [*10*]).

The tissues of the two species were stained simultaneously, in the same solutions. Mast cells were identified on the basis of their metachromatic granules. Their concentration in tissues was graded on a semiquantitative scale.

The results obtained by all staining methods were essentially identical and can be summarized as follows:

With the exception of the brain and the spleen—which contained no mast cells—the connective tissue in all other organs of the rat was constantly supplied with mast cells. These cells were extremely abundant in the thymus and the integument, numerous in the circulatory, respiratory, and urinary systems, and scarce in the digestive system.

By contrast, with the exception of the skin, the skeletal muscle, the heart, and the intestine—where very few cells with metachromatic granules were seen —we could find no mast cells in the other organs of the rabbit.

Recently, Bensley (*11*) introduced a new dye, alcoholic pinacyanol erythrosinate, which is a differential tissue stain and, in addition, selectively stains mast cells. Examination of 16 formalin-alcohol–fixed organs from each of two additional rats and rabbits with this excellent stain substantiated the results obtained by the previous methods.

In view of these findings, it appears possible that the great atherosclerosis susceptibility of the rabbit may be due to the rudimentary development of the heparin-producing gland in this species, and that the immunity of the rat may be caused by its much greater capacity for heparin secretion.

Records of species differences with regard to mast cells are contained in the earliest histological literature (*7, 10, 12*), although no systematic comparison of the rat and the rabbit has been made to date. Since no special significance could be attached to these findings in the past, and since the general trend has been to establish the mast cells as a constant component of vertebrate connective tissue, such contested reports as were made concerning the rabbit remained in the background. Thus, shortly after Ehrlich's (*13*) identification of the mast cells with metachromatic stains, his pupil Westphal (*14*) reported that mast cells occur abundantly in rats, bats, dogs, goats, and bovines but only sparsely in rabbits, birds, and cats. Shortly afterward, Raudnitz (*15*) reported that he could not find any mast cells in rabbits. Schreiber and Neumann (*16*), however, claimed that mast cells abound in some rabbit strains but are sparsely present in others. Also, von Möllendorf (*10*) and Michels (*12*) pointed out that rabbit mast cells are very difficult to demonstrate after aqueous fixatives and aqueous staining solutions.

It may be relevant to note that the rabbits employed in the present study have been verified to be highly atherosclerosis susceptible. Daily feeding of 1 g cholesterol (dissolved in 14 ml Mazola) produces macroscopic lesions after 5 weeks in this strain. Conversely, the rat strain studied proved refractory to comparable treatments.

The possibility that atherosclerotic or senile human subjects may present a mast cell deficiency similar to that of atherosclerosis susceptible rabbits is being investigated in this laboratory.

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