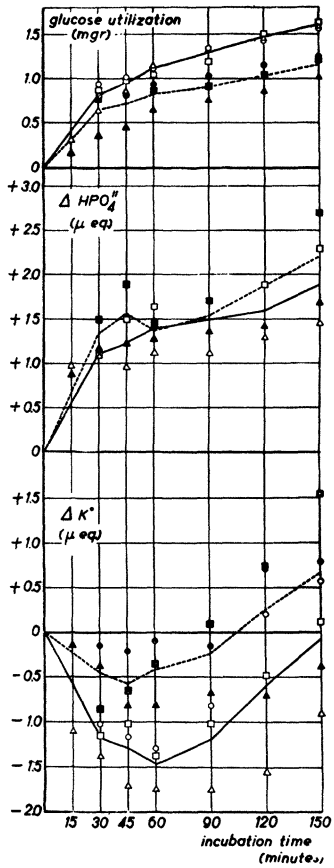

FIG. 1. Glucose utilization (in mg) and concomitant changes in inorganic phosphate and potassium content (in microequivalents) of the medium, calculated per 100 mg of diaphragm present in each vessel. The closed circles, squares, and triangles represent experiments without insulin; the averages are connected by the dotted curves. The open circles, squares, and triangles, the averages of which are connected by the continuous curves, represent the parallel experiments with added insulin.

containing in addition 0.5 units of insulin per ml.[2]

Both vessels were shaken at a rate of 120/min at 37° C, and samples of the medium were taken from each flask after 30, 45, 60, 90, 120, and 150 min. In the samples, glucose was determined by the method of Somogyi (*8*), inorganic phosphate after Briggs (*1*), and potassium with the aid of a flame photometer constructed by one of us (*10*). The results of three consecutive experiments, expressed as changes in the medium, calculated per 100 mg of wet tissue, are summarized in Fig. 1. Inorganic phosphate was determined in only two of these experiments.

Significant differences were observed in the glucose utilization, which proceeded at a greater velocity in the presence of insulin.

In the evaluation of the changes in the potassium and phosphate content of the medium, it should be kept in mind that every surviving tissue gives off phosphate and potassium through autolysis. This phenomenon tends to counteract any shift of phosphate or potassium which might accompany the penetration of glucose into the cells. Under these circumstances there was no evidence of a shift of inorganic phosphate into the diaphragms, either with or without insulin. The inorganic phosphate of the medium slowly increased in both vessels. The potassium content of the medium, however, showed a definite initial decrease, indicating a transfer of potassium into the muscle cells. In the presence of insulin the potassium shift was significantly larger in every experiment. The largest values for the potassium shift were found after one hour's incubation. At that moment, the difference in potassium shift between the flask with and that without added insulin amounted to about 1 microequivalent per 100 mg of wet tissue. It is concluded that a transfer of potassium from the medium into the muscle cells is associated with the utilization of glucose by the isolated rat diaphragm. This transfer is enhanced by the addition of insulin. Why we did not find in these experiments a concomitant shift in inorganic phosphate will be the subject of further study.

[2] We are indebted to Dr. J. Lens, of Organon Laboratories, for a crystalline preparation of insulin, containing 22.5 units per mg.

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The Effect of Changes in Body Weight on Atherosclerosis in the Rabbit[1]

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Atherosclerosis is probably responsible for more deaths on this continent than any other pathological process (*3, 4*). The high incidence of the fatal sequelae of this disease in the obese has been known to life insurance statisticians for many years (*1*). In 1947, Wilens (*9, 10*) reported analyses of autopsy findings in man which revealed atherosclerosis to be strikingly associated with adiposity. Evidence was also presented (*10*) showing that the characteristic lipid component of the lesions regresses during weight loss.

This evidence that a caloric intake in excess of energy requirement is associated with a high incidence of atherosclerosis in man suggests the possibility that weight loss due to decreased consumption or absorption of food,

[1] Supported by a grant from the Canadian Life Insurance Officers Association.

[2] The advice and encouragement of Dr. C. H. Best, under whose direction this work was performed, is gratefully acknowledged. We are indebted to Miss E. V. Gaston and Mr. G. W. Low for technical assistance, to Dr. Jean Patterson for help with the statistical computations, and to Mr. D. B. W. Reid for advice on statistical methods.

increased energy requirement, or urinary loss of energy-yielding metabolites might account for the efficacy of some procedures in preventing experimental atherosclerosis. We have investigated this possibility in the rabbit by examining the relationship between weight changes produced by various levels of food intake and the severity of experimental atherosclerosis.

The rabbits used were males of various breeds and of unknown age. They were housed in individual cages and allowed water ad libitum throughout the experiment. After a preliminary observation period of at least 1 week on Ralston Purina Rabbit Chow Checkers fed ad libitum, the animals were weighed and sorted on the basis of similar body weight into pairs or into groups of three. One animal in each group was chosen at random and allowed chow ad libitum throughout the experiment. The other member of a pair was offered 50% of the previous day's food intake of the freely fed animal. The other two members of the groups of three were restricted to 60% and 40% maximum intakes. The daily food consumption of each animal was recorded and all animals were weighed weekly.

After 2 weeks on the restricted feeding schedule (5 days in the case of rabbit 2—a replacement necessitated by appetite failure), the daily administration by stomach tube of an aqueous suspension of cholesterol was begun. The suspension was prepared by pouring cholesterol, dissolved in hot acetone, into cold water with constant stirring, and then boiling off the acetone and sufficient water to bring the concentration of cholesterol to 1 g in 20 ml of suspension. Each rabbit received 1 g of cholesterol daily, 7 days a week. Total cholesterol levels were determined on whole ear vein blood by the Schoenheimer-Sperry Method (*8*) before cholesterol administration was begun and weekly thereafter.

After 4–7 weeks of cholesterol administration the animals were killed and autopsied. The whole aorta was removed from each animal, fixed in 10% formalin, and stained with Sudan IV. The stained aortas were graded as unknowns into five arbitrary groups, according to the area of aortic intima involved by lesions. The presence of typical atherosclerosis was confirmed by microscopic examination.

Several animals were lost early in the experiment through damage to the respiratory tract during cholesterol administration. Only animals surviving 30 or more days of cholesterol administration are included in the series.

The data are summarized in Table 1. As expected, there is a highly significant correlation between food intake and final weight expressed as a fraction of initial weight ($r_{45} = 0.932$). The amount of intra-abdominal fat estimated by gross inspection at autopsy reflected the food intake.

In the absence of control of the other variables, the degree of atherosclerosis is not significantly correlated with cholesterol dosage, with initial weight, or with weight change. The correlation with average blood cholesterol is significant at the 5% level ($r_{Y2} = 0.430$).

Assuming that, within the ranges of the data, the correlations are linear, the effect of each of these independent variables on the degree of atherosclerosis, when the other independent variables are held constant, may be estimated by the calculation of multiple linear regression (*7*).

TABLE 1

Rabbit No.	Cholesterol dosage (g or days)	Avg blood total cholesterol (mg%)	Initial wt (kg)	Ratio of final wt to initial wt	Avg food intake per kg initial wt (g/day)	Degree of atherosclerosis
	X_1	X_2	X_3	X_4	X_5	Y
1	30	424	2.46	0.90	18	2
2	30	313	2.39	0.91	10	0
3*	35	243	2.75	0.95	30	2
4*	35	365	2.19	0.95	21	2
5	43	396	2.67	1.00	39	3
6	43	356	2.74	0.79	19	2
7	44	346	2.55	1.26	56	3
8	44	156	2.58	0.95	28	0
9	44	278	2.49	1.10	42	4
10	44	349	2.52	0.88	21	1
11	44	141	2.36	1.29	56	1
12	44	245	2.36	0.97	24	1
13	45	297	2.56	1.11	45	3
14	45	310	2.62	0.94	20	2
15	45	151	3.39	0.96	35	3
16	45	370	3.57	0.88	15	4
17	45	379	1.98	1.47	64	4
18	45	463	2.06	1.05	31	3
19	45	316	2.45	1.32	60	4
20	45	280	2.25	1.08	36	4
21	44	395	2.15	1.01	27	1
22	49	139	2.20	1.36	59	0
23	49	245	2.05	1.13	37	4
24	49	373	2.15	0.88	25	1
25	51	224	2.15	1.18	54	3
26	51	677	2.10	1.16	33	4
27	51	424	2.10	1.40	59	4
28	51	150	2.10	1.05	30	0

* Animals paired for ad libitum and 50% feeding after their respective restricted and freely fed companions died on the fourth day of cholesterol administration.

The coefficient of multiple correlation ($R_{Y.1234} = 0.7165$) is significant beyond the 1% point. The standard partial regression coefficients, indicating the relative significance of the independent variables in determining the estimated value of the dependent variable, are as follows:

$$b'_{Y1.234} = 0.150$$
$$b'_{Y2.134} = 0.553$$
$$b'_{Y3.124} = 0.484$$
$$b'_{Y4.123} = 0.535$$

The average blood cholesterol level, the initial weight, and the relative final weight are of approximately equal importance in determining the degree of atherosclerosis estimated from the equation of multiple linear regression. Under the conditions of this experiment, variations in the cholesterol dosage had little effect.

An association of severe lesions with high blood cholesterol levels has been noted by many investigators, as has, under some conditions, a relatively poor correlation

in the absence of statistical or experimental control of other variables (*4*).

Pollak (*6*) has reported an experiment indicating increasing severity of lesions with increasing age and body weight. Although in freely fed animals, not fully grown, a general parallel between age and body weight may be expected, we are unable to estimate the contribution of age in our results, since the exact ages of our animals were unknown.

The high net correlation found between relative weight gain and severity of experimental lesions is not only consistent with the findings in man (*9, 10*), but has an important application to the study of experimental atherosclerosis. In the evaluation of agents suspected of influencing experimental atherosclerosis in the rabbit, and probably in other species, there must be statistical or experimental control of changes in body weight. The low incidence of experimental atherosclerosis in rabbits rendered diabetic with alloxan prior to cholesterol administration (*2, 5*) is probably associated with the characteristic emaciation of these animals, rather than with a specific effect of the diabetes.

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Discovery of Dioecism in *Laboulbenia formicarum*

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Laboulbenia formicarum Thaxter is a very minute parasitic ascomycetous fungus that can be found very commonly on all exposed parts of the body of small red ants belonging to the genus *Lasius*. The species was originally described by Thaxter (*1*) from Cambridge, Massachusetts, and later shown in figures in Part II of his classical series of monographs on the Laboulbeniaceae (*2*). The two figures which represent the only ones Thaxter published of this species are both what appear to be mature female plants. Since no reference was made to antheridia of this species, either in the technical descriptions or in the brief notes, it seems reasonable to assume that antheridia and the plants on which they are formed were not observed by Prof. Thaxter and that he did not suspect the species of being dioecious.

During the fall of 1948, while collections were being made of parasitized individuals from a colony of red ants in Champaign, Illinois, a large number of males were obtained with wings still attached that were heavily infected with *Laboulbenia formicarum*. Plants were growing in great abundance on the wings (Fig. 1), and since these clear easily, plants in different stages of development, from the first stages in the germination of spores to mature plants, were readily observed. One of the first things to attract our attention when we started to examine this material was the way two plants were almost invariably associated (Fig. 2). A careful examination of such pairs where the perithecia of one were mature, as pictured by Thaxter, revealed that the smaller one which lacked a perithecium was not an immature or aborted individual but rather a mature male plant with well-defined antheridia. No antheridia were found on female plants and they were exactly as pictured by Thaxter. A study of younger plants has shown also that male and female plants are very much alike in early stages of their development, but even in early stages, after one has become familiar with the developmental sequence, males can be distinguished from females. The details of developmental morphology as observed in this species, accompanied by a series of figures, are being published elsewhere. The ascospores which give rise to male and female plants are discharged in pairs and cling together as they develop. A cytological study has never been made in any of the dioecious species of the Laboulbeniales of the phenomena involved in segregation of sex factors as

FIG. 1. Portion of the wing of a male ant heavily infected with *Laboulbenia formicarum*. Magnification × 150.

FIG. 2. A pair of mature individuals removed from the host. Magnification × 420.