THE GROWTH AND THE FOOD AND WATER CONSUMPTION OF THE RESTING OR EXERCISING ALBINO RAT ON DIETS CONTAINING VARIOUS AMOUNTS OF FAT AND A REDUCED QUANTITY OF THIAMINE.

II. PUBLICATION. SUMMER EXPERIMENTS.

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With 5 Figures and 2 Tables in the text.

(Received for publication 24th May, 1948.)

In an earlier publication (BEZNÁK, M. BEZNÁK, HAJDU 1947) we reported the growth of albino rats to be dependent on the fat content of the diet. On a diet containing a probably reduced quantity of thiamine, the growth of resting rats stopped if there was only 3% fat (in the form of sunflower-seed oil); above this value growth was good, tending to be proportional to the fat content. On forced exercise growth was resumed in the 3% fat group, it stopped in the higher fat-content groups, and a transitory loss of weight took place in the latter, proportional to the fat content of the diet.

To elucidate further the mechanism of this phenomenon, we wanted to decide: 1. Whether the proportion of fat in our diet had the same effect on growth throughout the whole year, or whether the same diets had different effects in different seasons. Thus, while our previous experimental period lasted from February 13th until June 13th, 1943, the present experiments were carried out between June 10th and November 10th, 1943. 2.) How long the effect of a given diet lasted; that is, how the changing of the diet from 3% fat content to 32% (and vice versa) at the beginning of exercise affected growth during that period.

METHODS.

Our experiments were carried out on 78 male albino rats of Wistar origin. The rats were of the same strain and sex, and almost the same age and weight, as those used in our previous experiments. They were divided in 6 groups, each group occupying a separate cage, constructed so as to make refection impossible. The weight of the rats was measured every third day in rest, and daily during exercise. Their food and water consumption was measured daily during the whole experimental period. Only 2 diets were used, both qualitatively identical, but with varying amounts of fat. The composition of the diets, which was identical with those described earlier, is shown in Table I.

TABLE I.

Composition of the foods consumed.

Sunflower oil	3%	32%
Starch	53%	24%
Casein	25%	25%
Spinach	10%	10%
Yeast	5%	5%
Salt mixture	4%	4%
g calories/kg	3650	5250
Total fat content, including the 0.5% casein-fat (Soxhlet)	3.5%	32.5%

For some physical and chemical properties of the sunflower oil used see our previous publication.

Group 1.: The animals in this group rested during the whole experimental period, thus living the life of the normal caged albino rat. The diet contained 3% fat.

Group 2.: These, after 44 days' rest, began a running period of one month, then rested till the end of the experiment. The diet contained 3% fat.

Group 3.: 44 days rest, 1 month running, rest to the end. This group consumed a diet containing 3% fat during the pre-exercise resting period. 7 days before the running began, this diet was changed to the one containing 32% fat, which it received to the end.

Group 4.: Resting group, receiving 32% fat.

Group 5.: 44 days rest, 1 month running, rest to the end. 32% fat.

Group 6.: 44 days rest, 1 month running, rest to the end. The diet of this group contained 32% sunflower oil during the pre-exercise resting period. 7 days before work was begun it was exchanged for the one containing only 3% fat, which it received to the end.

Work consisted in running in a treadmill, with a velocity of 1 km per hour. Running was increased from ½ hour on the first day, to 5 hours on the 13th day. This amount of work — 5 hours daily — was maintained till the end of the exercise.

EXPERIMENTAL RESULTS.

Figures 1—3 show the growth curves, food consumption and water intake of the 6 groups, constructed from the respective averages. Considering the growth of Groups 1 and 4, it can be seen from Figure 1. that it is slow in the group receiving 3% fat as compared to that getting 32%. At the end of the experimental period the difference, however,is only —7%. With the beginning of exercise (see Figure 2.) there is a marked loss in body weight in both 3% (No. 2.) and 32% (No. 5.) groups. The loss is small in the 3% group and is restricted to the first third of the exercise period, being preceded by a small rise during the first day after exercise. The loss in weight is greater and quicker in the 32% fat group. The loss shows a tendency to slow down at the end of the first ¼ — ⅓ of the working period.

If during rest, towards the end of the 44 days' resting period preceding exercise, the 3% diet is exchanged for one containing 32% fat (Group 3.), the rats immediately begin to grow faster. Accordingly a very sharp rise in body weight is observed in the curve of this group (Figure 3.). With the beginning of work, the weight immediately and rapidly falls as in the groups which consumed a 32% fat diet from the beginning. If, on the other hand, the diet of the resting rats, living on 32% fat, is exchanged for one containing only 3% (Group 6.), growth slows down. If work is now begun, some 7 days after the change in the diet, a sharp fall in body weight results. This fall runs parallel to the curves of groups receiving 32% fat, for about a week. After that time, however, the loss of weight stops and in the second half of the running period the rats begin to grow. (Figure 3.). Thus after the first week of exercise the rats behave like those getting 3% fat from the beginning of the experimental period.

To illustrate this point more clearly, the growth curves of the exercising groups are shows separately in F i g u r e 4. Taking the body weight on the last resting day of each group s equal to 0, the daily changes in gs. during and after exercise are plotted against time. All the points stated above are clearly visible from this graph.

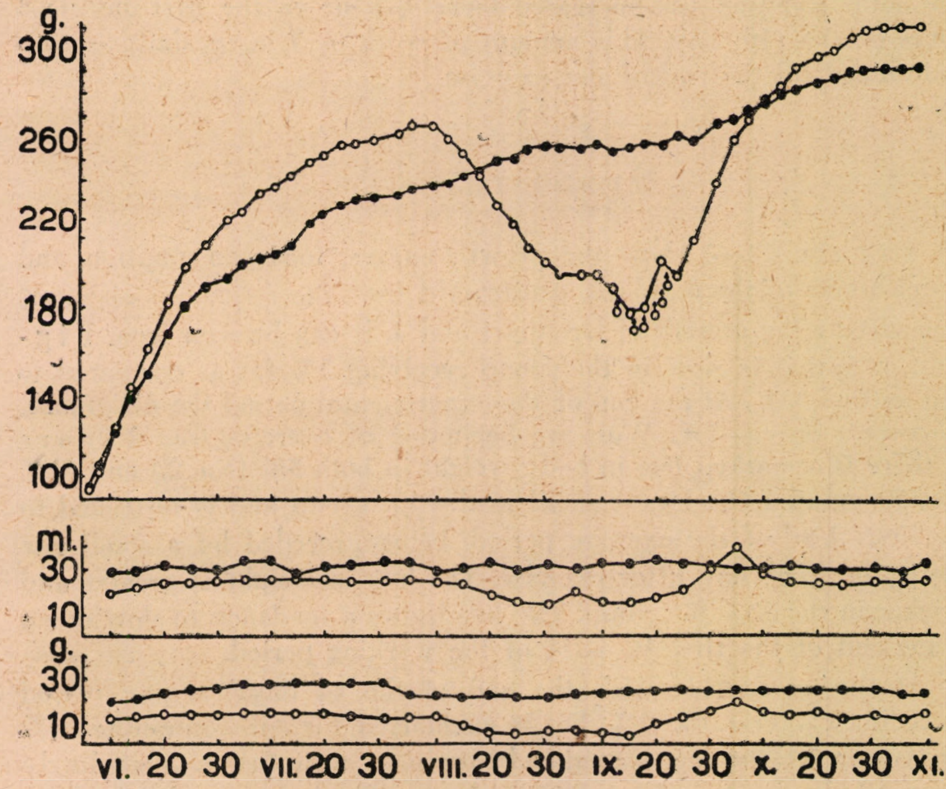

Figure 1. The growth, food and water consumption of the resting groups. Abscissa: experimental period in days. Ordinate, upper part: body weight in gs, middle part: water uptake in ml, lower part: food consumption in gs. ●——● 3% fat, Group-1, o——o 32% fat, Group-4.

F i g u r e s 1—3. also show the daily f o o d and w a t e r consumption of all 6 groups, in the three phases of pre-exercise rest, exercise, and post-exercise rest. The food and water consumption is inversely proportional to the fat content of the diet.

At the beginning of the exercise (Figure 2.) in both groups food consumption and water intake decrease, then during the last 3/4ths of the exercise there is a gradual rise in both. During the first quarter of the exercise period, when the body weight drops, the food consumption decreases simultaneously, whereas the water intake diminishes but little. From the end of the period of rapid loss in weight onwards, though there is no growth, the body weight remains constant,

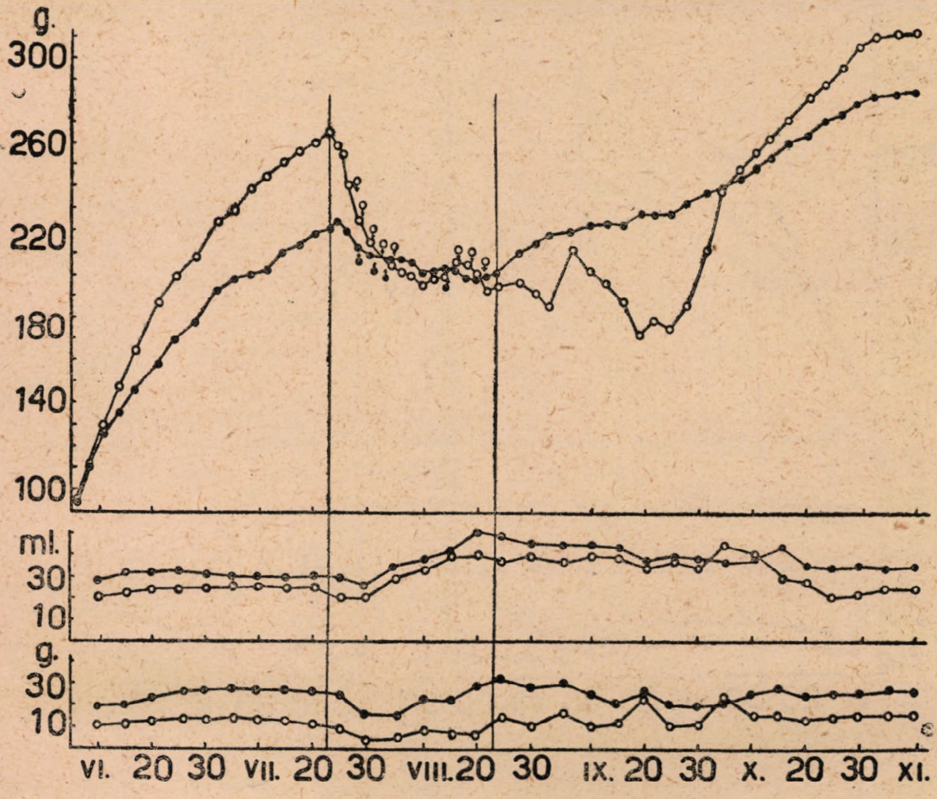

Figure 2. The growth, food and water consumption of the exercising groups. Abscissa: experimental period in days. Ordinate, upper part: body weight in gs, middle part: water uptake in ml, lower part; food consumption in gs. ●—— 3% fat, Group-2, o——o 32% fat, Group-5. Period of exercise indicated by the vertical lines.

but the water intake and food consumption increase considerably in both groups, exception being the food intake of the 32% animals, which rises slowly during exercise, but quickly after its cessation. It is interesting to remark that in the post-exercise resting period the 3%

animals begin to grow while their food and water intake slowly diminishes. The 32% animals, contrary to this, continue for some time to lose weight in spite of an increased food uptake, after which they suddenly begin to grow fast, without their food consumption being increased.

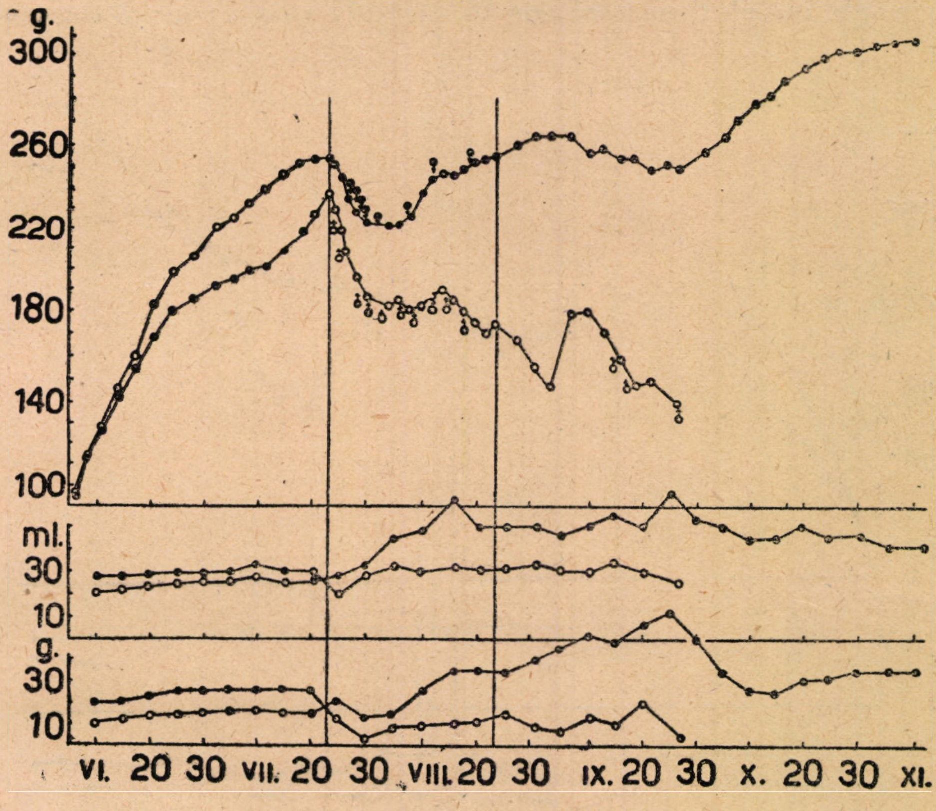

Figure 3. The growth, food and water consumption of the exercising groups where the fat content of the diet was reversed before the beginning of the exercise. Abscissa: experimental period in days, Ordinate, upper part: body weight in gs, middle part: water uptake in ml, lower part: food consumption in gs. ●——● 3% fat, Group-3, o——o 32% fat, Group-6. Period of exercise indicated by vertical lines.

In Figure 3. are shown the relationships between food consumption, water intake and growth during exercise of the animals where the fat content of the diet was reversed during exercise as compared with the pre-exercise resting period.

It is seen that increase in the fat content diminishes food consumption and so does exercise. Do these two effects add up when the 3% diet is exchanged for the 32% and the animals perform exercise? In the group eating 32% fat during the pre-exercise rest, the food consumption drops during exercise from 13g/day/rat to 6g/day/rat. In the other group, consuming 3% fat, the drop under similar conditions is

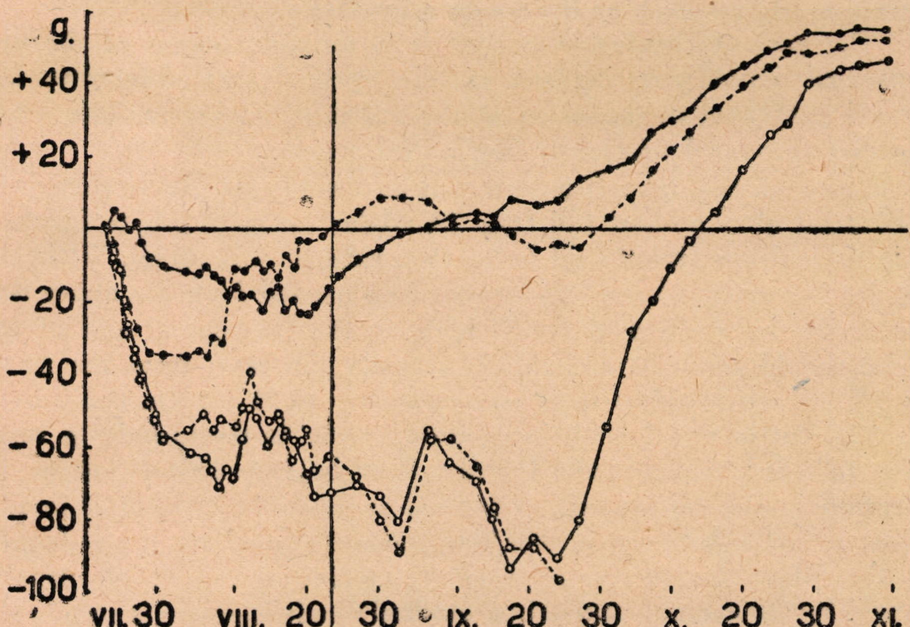

Figure 4. Growth of the exercising groups. Abscissa: experimental period in days, Ordinate: changes in body weight in gs. ●——● 3% fat group, o——o 32% fat group, ●---● 3% fat group which received 32% fat during the previous period of rest, o--o 32% fat Group which received 3% fat during the previous period of rest. Exercise lasted till the vertical line.

from 26 g to 16 g. When the food is changed from 3% to 32% and the rats exercise, the drop is from 26 g to 6 g. The combined effects of the change in fat content and of exercise in this case, therefore, do add up. Does that hold good for the reversed case? As described above, decrease in the fat content increases food consumption, exercise decreases it, and this effect is proportional to the fat content of the diet. When, therefore, the 32% fat diet is exchanged for the 3%, food consumption should rise, in our case from 13 g to 26 g. On the other hand, owing to the simultaneous exercise it should drop to 6 g if the animal consumes 32% fat and to 16 g if the fat is 3%. Now the animals eat 3% fat (during rest they eat 32%); food consumption during exercise should be 16 g. Actually the food consumption in the pre-exercise rest was 14 g, on lowering the fat content and in exercise it rose to 20 g, for the first 5 days, then dropped to 13 g. Here again, therefore, the two effects add up, i. e. from the rise in the food consumption caused by the lowering of the fat content, exercise deducts exactly as much as it does on a low fat diet.

The general conclusion is that it is the fat content of the diet consumed actually during the exercise period that determines the growth and food consumption of the animals and the fat content of the diet consumed previously has no influence.

As to water intake, it is seen that on the 3% fat diet it rises to 50 ml/day/rat after a short fall from 29 ml/day/rat to 26 ml; in the 32% animals it drops from 25 ml to 22, then rises to only 40 ml. When the 3% fat is exchanged for 32% it is to be expected from the above that the diminishing effect of the increase in the fat content and of exercise should combine, and this is actually the case: from the 28 ml on 3%, in rest, it drops to 21 and rises only to 32 ml. On exchanging the 32% fat in rest for 3% in exercise, owing to the exercise a small drop followed by a definite increase is to be expected; owing to the decrease in the fat content, a general increase in the water intake. What actually happens is that the increase caused by the reduction in the fat content over-compensates the initial depression usually caused by the exercise and only a gradual steady increase is seen in the water consumption which attains 50 ml/day/rat. This is the same level reached during exercise by the rats consuming 3% fat during the pre-exercise rest too. In respect to water consumption, therefore, the same rule already observed in the case of growth and food consumption holds good.

During the post-exercise rest period there is yet another loss in weight in all the six groups. It occurs at the same time (the first 3 weeks in September) in all the groups. It is considerably greater in the animals consuming 32% fat (90—100 g) than in those eating 3% fat (only a few gs). Within these groups it is greater in the animals which have exercised than in the resting ones, and of the 2 exercising groups it tends to be greater where the fat content was changed from 3% to 32%. The loss of weight, therefore, increases in the following order: resting 3% → exercising 3% → 32% during rest 3% during exercise → resting 32% → exercising 32% → resting 3%, exercising 32% animals.

The mortality (shown in the figures as ♂ or ♀) alone remains to be described. Only in the 3% resting group no mortality occurred; in the other 5 groups there was a fairly high mortality. In the exercising groups it took place during the period of exercise, in the resting 32% group at the end of the September loss in weight. The frequency increased as follows: 3% exercising (4 cases) → resting 32% (6 cases) → exercising 32% and resting 32%, exercising 3% (8—8 cases) → 3% rest, 32% exercise (13 cases). It is clear from this that high fat content in the diet and exercise both increase mortality.

DISCUSSION.

The first question is whether any difference in the growth response, food consumption or water intake of the two (spring and summer) sets of animals can fairly be attributed to the change in the season. The diet consisted of the same materials as used during the spring and it was prepared in exactly the same way. Unless during storage some change took place in the composition of the basic materials, the diets are to be regarded as identical. The other difference, namely that in the animals themselves, is also minimized by taking animals from the same inbred colony, the next or next but one generation of the same mothers of the same sex and almost the same age.

Elsewhere (BEZNÁK, M. BEZNÁK, GÁSPÁR-RÁDY, 1947) the importance of age in nutritional experiments has been pointed out. It was shown there that, according to the relation, the post-natal life-span of the albino rat is to be divided into five phases. Though our summer animals were slightly younger than the spring ones (64 and 75 days respectively) the pre-exercise rest and the exercise fall into the period we called 3 b. When there is a similarity between the changes in the two groups (spring and summer) it verges on identity, when there is a difference it is very marked. The conclusion, that the differences are due to to the effect of the difference in the seasons therefore appears to be warranted.

In conformity with our spring results, during summer the growth, food and water consumption in rest and in exercise are dependent on the fat content of the diet. In the spring, on the 3% fat ration, growth stops during rest, and is resumed in consequence of exercise; in the summer it only slows down in the rest and there is a loss in weight during exercise. On the 32% fat ration both in spring and in summer, growth is faster during rest and there is a loss of weight during exercise, but this is less during the former season. The loss in weight of the 3% summer animals during exercise is much smaller than that of the 32% ones. In fact the 3% summer animals behave exactly the same during exercise as do the 16% animals in the spring. The weight of the former drops from 233 g to 220 g, that of the latter from 220 to 205 g. The two growth curves during rest are also almost identical, inasmuch as the animals of both seasons grew to 230 g during 44 days rest.

Though the summer animals grew somewhat faster, during the 44 days pre-exercise rest, they built up some 25 g more body weight than the spring animals did.

To turn to the 32% fat group: In spring it was found that during rest growth tends to be proportional to the fat content, and during exercise the loss of weight is definitely proportional to it. In the spring the 32% animals reach 235 g during the 44 days' rest, in the summer 265 g. Summer, therefore, enhanced the growth-promoting effect of fat. The animals grew as fast as if the fat content had been greater than 32%. The loss of weight during the spring is from 235 g to 215 g; during summer from 265 to 190 g. Thus summer also increases the loss of weight seen in exercise, in proportion to the fat content. In this case the effect of summer is again an increase in the response to the fat content. The growth of the 32% group during the summer shows the same change, as compared to the spring experiment, as the 3% group, i. e. as if the fat content of the diet had been greater than it actually was. From the above the conclusion seems warranted that the growth response of the organism to the fat content of the diet is essentially the same both in rest and in exercise, in spring and in summer, but that the response is considerably enhanced in summer. Thus, in spring the organism responds to one-fifth the fat content required to bring about the same response in summer.

As in our spring findings, the food and water consumption in summer is in inverse proportion to the fat content of the diet. The food consumptions of the 32% and 3% groups, in spring and summer, during the pre-exercise resting period, are compared in Table II. In both groups food consumption is higher in summer than in spring. The difference is greater (almost +30% of the spring consumption) in the 3% animals, than in the 32% ones, where it amounts to only 10%. In the spring the food consumption is inversely proportional to the fat content of the diet, as it is also in summer. But during the spring the maximum food consumption (in the 3% group) is 19.0 g and the minimum (in the 32 group) is 11.9 g. The 7.1 g difference between the two is 60% of the minimum, 13.1 g. The 11.5 g difference between the two 32% groups is 88% of the minimal consumption. As to growth, it was seen that in rest summer enhances the growth-promoting effect of the fat content, in exercise its weight-decreasing effect. The summer 3% animals behave in this respect like the 16% spring ones. In the case of food consumption, the effect of the increase in fat content in spring was a depression in food consumption. By analogy to the growth effect of summer, one would expect a decrease in the food consumption both in the 3% and 32% groups. Whereas the difference between the 3% and 32% food consumption in the spring is only 60%, in summer it is 90%. The correct conclusion appears to be that in summer for some cause the food consumption is higher and the depressing effect of the fat on food consumption — like its effect on growth — is also greater during summer than in spring. But since this acts against a food consumption on a considerably higher level, the net result in gs is an increased food consumption in summer.

During exercise in the spring in the 8, 16, and 32% groups, there is a diminution in the food consumption proportional to the fat content of the diet. Contrary to this, in the 3% animals food consumption

TABLE II.

The daily food consumption/rat during the pre-exercise resting period.

	3% fat content		32% fat content	
	Summer	Spring	Summer	Spring
No.	40	43	40	43
M.	24.6	19.0	13.1	11.9
μ	2.9	1.2	0.9	1.9
	k = 11		k = 3.7	

No. = number of days
M. = average daily food consumption per rat.
μ = standard deviation
k = significance of difference

increases. The food consumption of the present summer animals during exercise decreases in both the 3% and 32% groups. In discussing growth it was pointed out that on a 3% diet the summer animals grow both in rest and in exercise as the spring animals do on 16% fat diet. The circumstance that in the summer animals a more pronounced effect on growth is exercised by the fat than in the spring, was also found to be true in the resting food consumption, where, though there was a general increase, the greater fat content caused a greater diminution than in the spring. It follows from this that in the summer, during exercise, in the 3% group a loss and not a gain, and in the 32% group a greater loss, is to be expected in the food consumption. As has been seen above, this is actually the case here. Therefore, as in the other cases considered so far, the fat is also more effective here in summer.

It was suggested in our previous paper that the inverse proportion between food and dietary fat during rest, and its further depression during exercise, is not due alone to the high calorific value of the fat, thus a smaller quantity satisfying the energy requirement, but also to the effect of some intermediary metabolites of the fatty acids. The difference between the summer and spring animals must, therefore, be due either to a faster fatty-acid metabolism or to a greater sensitivity of the summer organism to these intermediary metabolites.

As regards the water intake, it was seen that in the spring animals this increases in consequence of the exercise and that the greater the fat content, the later the increase reaches its maximum. In the 3% and 8% animals, in the spring, the maximum is reached on the 23rd day of the exercise, in the 16% group it is some 8 days later (2—3 days of which fall within the post-exercise rest), and in the 32% animals there is a delay of some 12 days and the maximum is 6—7 days after the exercise has already ended. If these effects of the fat are also increased in summer, the increases in the water intake should be shifted in proportion to the fat content, towards the end of the exercise. This, actually, does not happen. Summer shifts the increase in water intake into the first third of the exercise in both 3% and 32% groups. Here, therefore, the increased effect of fat in summer does not operate.

The increase in water intake was attributed in our previous work to an increase in the protein and salt metabolism due to the exercise. Its earlier onset in summer animals would indicate an earlier increase of these metabolisms.

In order to gain some information about the sequence of changes in the growth, food and water consumption consequential to the beginning of exercise, the effect of the season and the fat content of the diet, diagrams were made in which these changes are recorded daily.

It is to be seen in Figure 5 A. that in the 3% spring animals the resumption of growth starts on the 3rd day of exercise, whereas food consumption begins to increase only on the 5th day.

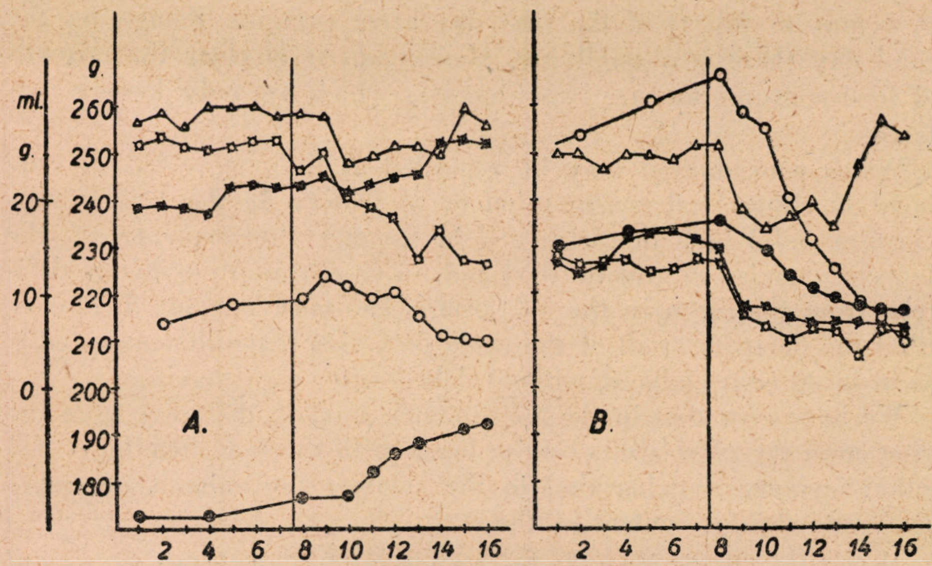

Figure 5. Daily changes in growth, food and water consumption in the 3% and 32% summer and spring groups. Abscissa: days; First ordinate: food in gs. and water in ml; Second ordinate: body weight in gs. A: 3% fat groups, B: 32% fat groups. o——o growth of summer groups, ●——● growth of spring groups. ¤—¤ food consumption of summer groups, ●——● food consumption of spring groups, △—△ water intake of summer groups. Exercise begun at the vertical line.

It is interesting to remember that HOPKINS (1912), when he discovered that a small addition of milk to a synthetic diet restores growth in rats, pointed out that growth is resumed first and that increases in the food consumption follow. In our case where not the addition of the missing dietary factor, but exercise, causes the resumption of growth, the sequence of events is the same.

It was suggested in our first paper that the mechanism of the resumption of growth might be the synthesis of some indispensable intermediary metabolite of the fatty acid metabolism formed from the carbohydrate intermediary metabolism increased by exercise. Perhaps the circumstance that growth resumption and food consumption follow each other as they do, when a dietary deficiency is cured by the addition of the missing indispensable factor, may be regarded as an indirect support of our hypothesis.

In summer in the 3% group exercise is followed during the first 4 days by a very small rise in growth. In spite of this, food consumption begins to fall from the first day after exercise, though on the second day there is a small rise. Here again it is clear that growth and food consumption are two processes which are only loosely correlated.

Water consumption may be regarded as even more loosely correlated to either food consumption or to growth. In the 32% groups (Figure 5 B.) both in spring and in summer the sequence of the effects of exercise on growth, food and water consumption differs definitely from that seen in the 3% group. The most obvious difference is that the decrease in all of the three processes is simultaneous and it sets in in 48 hours (almost without delay) after the exercise.

We have seen that in the 3% summer animals, differing from the spring ones, the effects of exercise begin with the same readiness. Yet another instance showing that in the summer the animals are more sensitive to the fat content of the diet.

In our first paper we gave the reasons why we attribute the loss in weight in exercise on a diet rich in fat to water loss. This water loss is supposedly caused by some intermediary metabolites of the fatty acids, the concentration of which is increased in consequence of the increase in the fatty acid metabolism, this latter being due to exercise. In summer the loss in weight is greater and lasts longer than in the spring. There are two possible explanations: in the summer either the fat metabolism is greater, or the body is more sensitive to the intermediary metabolites of the fatty acids.

As shown in our first paper, in the spring and in rest, growth of the 3% fat group is stunted and exercise restores it without the addition of either fat or vitamin B_1. This curing effect of exercise was suggested to be due to the synthesis of some semi-indispensable fatty acid (or its intermediary metabolite) synthetised from the increased intermediary carbohydrate metabolites. In the summer we see that the 3% animals grow as well as the 16% do in the spring; also during exercise there is no improvement in the growth, but a loss in weight. The effects of the changes in diet, the behaviour of the food and water consumption, their changes in exercise all unanimously indicate that during summer fat has a greater effect than in the spring. All this points to an increased metabolism in the summer animal. Thus in such animals consuming only 3% fat the alleged synthesis of the semi-indispensable fatty acid from carbohydrate intermediary metabolites may be greater in rest than it is in spring, hence the better growth. In other words, summer has the same enhancing effect as exercise in spring. When the 3% summer animals lose weight in consequence of exercise this is possibly due to the balance between production of the semi-indispensable fatty acid and an increase of fatty acid metabolites being shifted into the latter direction.

In closing a few words must be said about the spontaneous loss of weight and mortality between August 20th and the end of September. It is to be emphasized that these are characteristics of the summer animal, and that either exercise or high dietary fat are necessary for its manifestation. Its cause is unknown; it may be some infection, or some metabolic breakdown. Whatever its causes, it proves the greater susceptibility of the summer animal.

SUMMARY.

1. The growth, food and water consumption of albino rats in rest, forced exercise and rest following exercise, on diets poor in vitamin B_1 and containing respectively 3 and 32% fat, were established during the summer, and compared with earlier spring results.

2. In rest throughout the span of growth between 100 and 300 g, during summer, growth is slower on the 3% than on the 32% diet, though it is greater in both groups than it is in the corresponding spring groups. The summer increase is considerably greater in the 32% than in the 3% group.

3. In the summer, during forced exercise, both groups lose weight, but the 32% more than the 3% group. In the spring, exercise restored and maintained growth which had been stunted during the pre-exercise rest on the 3% diet. The loss of weight in the 32% group during summer is considerably greater than in the spring.

4. Food and water consumption during the summer — in animals resting throughout the entire growth span — is inversely proportional to the fat content of the diet. This inverse proportion was seen in the spring experiments also. Summer animals, however, consume more food

in all groups than the spring ones do and this increase in the summer consumption is also inversely proportional to the fat content of the diet. In summer, during exercise in both 3% and 32% groups, food and water consumption decrease. The decrease is greater in the latter than in the former group. In the spring there was an increase in the food consumption of the 3% group. In the 32% group during exercise, both in spring and in summer, food and water consumption decrease at the beginning of exercise. This is followed by a recovery in both cases in the last 2/3rds of the exercise period.

5. When, during summer, after 44 days of pre-exercise rest, the fat content of the diet for the exercise period is reversed from 3% to 32% and vice versa, growth, food and water consumption behave according to the fat content of the diet consumed during exercise. The effect of the increase in the fat content is instantaneous, that of the decrease has a few days' lag. In the animals whose resting 32% diet was exchanged for the 3%, increase in the water consumption, from the last 2/3rds of the exercise period onwards is greater than in the other groups.

6. At the end of the summer, parallel with a great mortality, there is a long and great spontaneous loss in weight, in all animals except the ones resting on the 3% diet throught the summer. In the summer animals mortality also occurs during exercise. Mortality and loss of weight increase with the fat content and exercise. These phenomena were completely absent in the spring animals.

7. It is suggested that the effect of fat and exercise on the growth, food and water consumption is essentially the same in summer and in spring, but in the summer all effects of fat are greater.

Grateful acknowledgment is made to Mrs. Jean Thompson Vass for looking through the English of this paper.

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