Studies on Genetically Determined Metabolic Patterns

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SOME TIME AGO we made the observation that under suitable conditions of maintenance the feeding of synthetic rations which are very high in fat could induce obesity in some strains of mice while leaving at least one strain lean.[1-3] In the most susceptible strains there is a linear relationship between dietary levels of fat and the amount of carcass fat deposition. In these susceptible strains one observes a sharp decrease in the rate of formation of the fat-free component while carcass fat increases linearly to five months of age. In the I-strain mouse the formation of both fat and fat-free components diminishes at two to three months of age. The result is a sharply reduced rate of weight gain and of the maintenance of lean carcass composition to at least six months of age. A variety of experiments have demonstrated that to a considerable extent the obesity induced by the feeding of a diet high in fat content can be accounted for on the basis of increased caloric intake with increase in caloric density of the diets. However, differences in activity[4] and in oxygen uptake[5] have been observed.

The existence of strains of mice differing so sharply from each other in their regulation of food intake led us to examine some of the underlying metabolic and endocrine patterns.

When A- and I-strains of mice were pair-fed a diet high in fat content[6] for a period of forty-eight days following weaning, more fat was deposited in mice of the A-strain than in those of the I-strain. This suggests the existence in the A-strain mouse of facilitated channeling into the fat depots, which may help to explain the failure of this strain to restrict food intake while on rations of high caloric density since rapid deposition of ingested fat may serve to reduce the intensity of the satiety signal.

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FAT MOBILIZATION

Another interesting aspect of fat metabolism has been the mobilization of fat to the liver during fasting.[7] The levels of fat in the liver in the fed state are the same for A- and I-strains of mice; however, large amounts of fat are mobilized to the liver in mice of the A-strain when they are subjected to a forty-eight-hour fast (Fig. 1). The I-strain mouse subjected to the same fast shows an almost imperceptible rise in levels of fat in the liver. This observation might be the result of slow mobilization of fat; it might also be the result of rapid oxidation of fat in the liver of the I-strain mouse. Since both the A- and I-strains of mice used in this experiment had been raised on a low-fat commercial ration, their body weights and fat content were about the same. Therefore, it cannot be argued that mice of the I-strain had less depot fat to mobilize.

In the experiment reported in Figure 1, forty-eight-hour fasts were initiated at different times during the day with different groups of animals. The increase in levels of fat in liver of mice of the I-strain between eight in the morning and two in the afternoon is statistically significant. It is probably due to a marked reduction of food intake of this strain during the daylight hours. The rise in levels of fat in the liver could be prevented by oral ad-

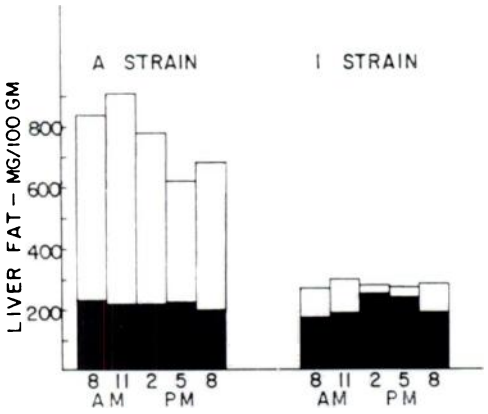

FIG. 1. Levels of liver fat (mg. fat/100 gm. initial body weight) of fed mice (solid bars) and forty-eight-hour fasted animals (open bars).

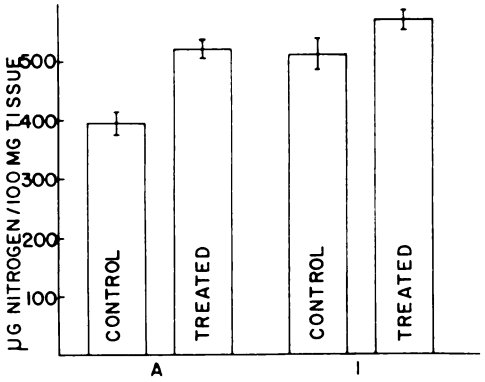

FIG. 2. Total nitrogen released by mouse diaphragm during three hours of incubation.

ministration (stomach tube) of a substantial dose of carbohydrate. The mice of the A-strain showed no such changes in levels of fat in the liver.

NITROGEN RELEASE

A variety of experiments demonstrated that turnover of protein in the I-strain mouse is significantly greater than it is in the other strains.[8] In a typical nitrogen balance experiment it was found that, at all levels of nitrogen intake studied, the nitrogen excretion of the I-strain mouse was significantly greater than that of the A-strain mouse. In a related experiment at the tissue level it was observed that the release of nitrogen, using the isolated diaphragm technic, was greater for tissues taken from animals of the I-strain (Fig. 2). This technic[9] involved measurement of the total amount of nitrogen released during three hours of incubation in a Krebs-Ringer solution.[10]

The greater turnover of protein in mice of the I-strain suggested that amino acids or other nitrogenous compounds might exist in large amounts in circulation and thereby contribute to the sensitivity of the regulation of food intake of these animals. However, the ultramicro methods required for repeated determinations of amino nitrogen and urea on 20 μl. quantities of tail blood have just been perfected. In the meantime an indirect approach to this problem was taken. On the supposition that increasing the level of dietary protein would increase circulating non-protein nitrogen, we increased the level of dietary protein from 30 per cent to 90 per in stages. This caused an irregular but marked decrease in the food intake of mice of the I-strain while comparable mice of the C3H strain were not affected.[6] Conversely, decreasing the level of dietary protein from 15 per cent caused a rise in food intake in all strains.

Efforts have been made to study certain aspects of carbohydrate metabolism. We have found no large differences in glucose tolerance, although the mice of the I-strain show somewhat better tolerance than the others. On the other hand, the levels of fasting blood sugar of the mice of the I-strain were consistently higher. Within each strain there was an increase in fasting blood sugar with increasing body weight.[11] The I-strain mouse showed extraordinarily high levels of muscle glycogen, but the levels of liver glycogen were lower than in the other strains.[12] *In vitro* studies showed isolated diaphragm and abdominal muscle of the mice of the I-strain to possess considerable glycogenolytic activity.

ENDOCRINE PATTERNS

Some tentative efforts have been made to modify the endocrine balance in the several strains of mice in the hope of throwing some light on the relative output of hormone. In the work on nitrogen release using the aforementioned isolated diaphragm we have compared not only tissues taken from normal untreated animals, but also those tissues taken

from mice injected with one of several hormones. In these experiments, at the dosages used, only thyroxine had a significant effect.[10] Daily treatment with large doses of thyroxine for seven days led to a large and significant increase in the release of nitrogen by the diaphragms of the mice of the A-strain. Similar treatment produced only a small and statistically insignificant increase in the release by I-strain tissue. It is tempting to suggest that these data indicate that the I-strain mouse operated normally at a relatively higher output of thyroxine than did the A-strain mouse.

CARDIAC GLYCOGEN

The mobilization of fat to the liver during fasting seemed so much more pronounced in mice of the A-strain that it suggested a greater output of the growth hormone in this strain. Efforts were made to utilize changes in cardiac glycogen during fasting or after administration* of the growth hormone as an index of the relative output of the growth hormone. Progress in this direction was hampered since neither fasting nor administration of the growth hormone caused any increases in cardiac glycogen; fasting for forty-eight or seventy-two hours actually caused a decrease. To test the effectiveness of our technics and the potency of our preparation of the growth hormone we repeated these experiments with rats and observed the changes in cardiac glycogen which was reported by Adrouny and Russell[13] and others. Similar increases in cardiac glycogen were obtained in the mouse only when the experiments were conducted at the temperature of 30°c. This rather elevated temperature maintains the mouse within a few degrees of its critical temperature of 33°c. At ordinary laboratory temperatures the rat also is close to its critical temperature of 27°c. Similar dependence on environmental temperature has been observed in some experiments on the role of adrenocortical steroids in protein catabolism. We have been able to show a significant drop in the adrenal ascorbic acid mouse when animals are moved from a room at 30°c. to one at 23°c. for a period of four hours. However, even at 30°c. the I-strain mouse showed no increase in cardiac glycogen during fasting, supporting the concept of relatively poor ability to release the growth hormone.

Many aspects of the nitrogen metabolism of mice of the I-strain suggest greater adrenocortical activity in this strain, however, some of our own observations are not compatible with this interpretation. Similarly, it is tempting to explain the high levels of muscle glycogen which occurred in mice of the I-strain on the basis of a greater output of insulin, but there are strong arguments against this. In order to define precisely the endocrine patterns of our four strains of mice, more direct measurements of hormone output must be made.

SUMMARY

Genetically determined patterns of carbohydrate, fat and protein metabolism have been observed. To some extent these patterns have been related to genetically controlled rates of hormone output.

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DISCUSSION

DR. HERBERT S. ANKER (*Chicago, Illinois*): Have you crossed the mice of strains A and I? What happens?

DR. FENTON: We have done this. We did it some time ago when we were still engaged principally in nutritional studies. We applied certain very elementary criteria, such as nitrogen excretion on nitrogen-free rations to measure the endogenous nitrogen output, and the deposition of fat on high-fat diets to crosses and back-crosses of these strains. We discovered that the genetic factors which determine nitrogen metabolism and those which determine deposition of fat in the carcass are not the same. There may be some overlap, but the inheritance definitely does not coincide in the two systems.

DR. DWIGHT J. INGLE (*Chicago, Illinois*): In the 1930's an extensive series of studies was carried out on rats by Palmer and Kennedy at the University of Minnesota, who developed high- and low-efficiency strains by selective breeding. These studies were not well known because most of them were published in bulletins of the School of Agriculture. At that time, they did quite thorough studies, attempting to determine the reasons for the differences in efficiency of food utilization. However, the methods were not then adequate to reveal the reasons for the differences. I think that today our methods might give an answer why one strain can gain much more weight on a fixed amount of food than can another.

DR. FENTON: We have some measurement of physical activity in our strains of mice. On low fat rations, the mice of strain I are much more active, as might be anticipated. On high fat diets, the other strains tend gradually to increase activity while they are increasing their caloric intake. But, oddly enough, strain I reduces its activity in high fat feeding, which is very perplexing.

I hope, with the system we are now developing, that we can measure simultaneously activity and uptake of oxygen along with food consumption. I think all three need to be known.