Changes in Body Composition and Respiratory Quotient of Adult Female Rats treated with purified Growth Hormone

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There have been numerous demonstrations of the existence in the anterior lobe of the pituitary of a hormone which, when injected into adult, fully grown rats, is able to cause a resumption of somatic growth. The increase in body size is not simply a resultant effect of a stimulation of appetite, since both the results of Lee & Schaffer (1934) and the more recent experiments of Young (1945) have demonstrated that animals treated with anterior pituitary extracts gained in body weight even when their food intake was limited to that of normal control animals. The mechanism whereby rats treated with growth-promoting pituitary extracts, are able to increase in weight and deposit extra protein in their tissues, on a diet identical in composition and quantity with that which will only just maintain similar animals not treated with growth hormone in weight stasis and nitrogen equilibrium, has not been adequately explained.

The results of carcass analyses obtained by Lee & Schaffer (1934), Young (1945) and Li, Simpson & Evans (1948) have shown that the deposition of protein in the bodies of rats treated with growth-promoting anterior pituitary extracts, is accompanied by an increase in water and a decrease in fat and carbohydrate content, and since there is also a growth of the long bones, this process has been characterized as 'true growth' (Li, Simpson & Evans, 1949).

The changes in body composition following hypophysectomy were the converse of those occurring in growth-hormone-treated animals. Hypophysectomized rats lost more nitrogen and less fat than did intact, pair-fed control animals (Li *et al.* 1948), and if young hypophysectomized rats were forcibly fed the same amount of food as that eaten by normal control rats, it was found that they stored less nitrogen and more fat than the control group (Samuels, Reinecke & Baumann, 1943; Levin, 1944).

A parallelism therefore appears to exist between the deposition of proteins in the tissues under the influence of anterior pituitary growth hormone and the catabolism of fat. The experiments described below were designed to show that this parallelism is not fortuitous, but that the increase in body protein is effected at the expense of the oxidation of fat, and as a corollary, an increment in weight can only be achieved while there is labile neutral fat which can be oxidized for energy purposes.

EXPERIMENTAL

Animals. The rats used in these experiments were fully grown adult females of the Medical Research Council hooded Norway strain, aged 5 months and weighing between 180 and 200 g. They were maintained on the special diet used in this laboratory for paired feeding experiments, the composition of which is: 85% extraction flour, 1090 g.; dried skimmed milk, 150 g.; casein, 150 g.; brewer's yeast, 260 g.; beef fat, 60 g.; cod-liver oil, 13 g.; $CaCO_3$, 22 g.; water, 800 g. The diet had a protein content of 15·5% and a fat content of 2·85%; it yielded 260 kg.cal./100 g. (calc.).

In a preliminary experiment, the average daily amount of diet eaten by a group of twenty-four rats over a period of 14 days was 22 g. This amount of food was therefore fed as the daily ration for the controls and for one group of the growth-hormone-injected animals, the 'limit-fed group'. The second group of growth-hormone-injected rats was allowed excess food (30 g.) and permitted to eat as much of this as they required; this was the 'free-fed group'. The animals were randomly selected and allocated to the various groups so that in the free-fed group each point represents the mean value of three rats. In the limit-fed group each point represents two rats and in the control group each point represents one rat. The small number of rats in the latter group is justified by the uniformity of values found over the whole experimental period.

Hormone preparations. The growth hormone was a preparation of the crystalline hormone from beef pituitaries, the 'fraction A' prepared according to the method of Wilhelmi, Fishman & Russell (1948). Wilhelmi *et al.* (1948) found less than 0·05% of adrenocorticotrophic hormone (ACTH) in this preparation, so that at the maximum dose level used here not more than 0·6 μg. of ACTH was given as contaminant. For injection the hormone was suspended in water and the pH adjusted to 8·0 to ensure complete dissolution. The volume was then adjusted so that 1 ml. contained 1 mg. of the hormone. This was injected subcutaneously at the rate of 0·25 mg./day. The rats on long-term treatment had their dosage stepped up to 0·5 mg./day after 30 days, and those in the two groups treated for 60 and 70 days respectively had a further increment to 1 mg./day after 50 days. Controls were given the appropriate volume of saline. Injections were always given between 9 and 10 a.m. The animals were weighed and fed at the same time.

Determination of respiratory quotients. Measurement of the respiratory quotient (R.Q.) of the intact animals was made in an apparatus modified from that described by Morrison (1947). The original apparatus described measured the O_2 uptake only by determining the amount of O_2 which had to

be admitted to the apparatus during the course of the experiment to keep the volume constant. This apparatus was modified to include three absorption tubes containing $Ba(OH)_2$. The rat was maintained in the apparatus on open circuit until it became quiescent, after which the apparatus was closed and the $Ba(OH)_2$ tubes introduced into the circuit by means of a two-way tap. The O_2 uptake was measured as described above and the CO_2 was measured gravimetrically after absorption by $Ba(OH)_2$. The duration of the determination was fixed as the time necessary for the rat to absorb 130 ml. O_2 (at room temperature and pressure); these conditions were selected because the time was convenient and the volume easily measured on the respirometer used. In forty determinations the time was between 22 and 31 min. with a mean value of 26·2 min. Throughout the period the rat was kept under observation and the determination abandoned if the rat showed signs of agitation. The measurements were always made 24 hr. after the last feed in order to minimize the variations described by Werthessen (1937).

Measurements of the R.Q. of liver slices and of diaphragm were made by the method of Dickens & Šimer (1931) using Dickens & Greville (1933) flasks. The liver slices, 0·4 mm. thick, were cut on the microtome described by Stadie & Riggs (1944). The diaphragm was removed from the animal, the fat and the thick outer margin trimmed off, the central tendon was quickly removed and the remaining tissue used without further preparation. The medium was the bicarbonate-Ringer of Krebs & Henseleit (1932) and the gas phase a mixture of 95% O_2 and 5% CO_2 (v/v). The R.Q. was measured over a period of 3 hr. in the absence of any added substrate, as well as in the presence of 0·02 M glucose or 0·02 M sodium acetate (final concentration). Small samples of both liver and diaphragm were taken for dry-weight determinations.

Carcass analyses. Carcass analyses of the rats were carried out as follows. Since it was found impossible to obtain a uniform mince of the rat when the skin was included, the first step in the procedure was to skin the animal and weigh body and skin separately. The skinned carcass was then thoroughly minced in a power-driven mincer and a sample of the product (about 10 g.) was taken for moisture determination by drying at 104° to constant weight. In order to prevent the loss of fats the sample was enclosed in a fat-free filter paper cone, which lined the inside of the crucible and effectively absorbed any molten lipid. The skin was pinned, fur downwards, to a cork mat, dried for 6 hr. at 104° and then reweighed. The dried parchment-like skin was then frozen hard and broken into small fragments. A sample of the dried skin, proportionate in weight to the sample of carcass taken for moisture analysis, was taken and added to the dried carcass sample. These together, in the same proportions as wet skin weight to skinned carcass weight, were exhaustively extracted with light petroleum, boiling point 40–60°, in a Soxhlet apparatus. From the weight of lipid extracted it was possible to calculate the total fat content of the rat. The nitrogen content was measured on a similar composite sample by micro-Kjeldahl.

RESULTS

Growth curves and carcass composition

Growth curves for the three groups of rats are shown in Fig. 1. The curves are plotted as the percentage increase on the initial body weight against time to allow for the different initial weights of the individual rats. Each point on this graph represents the mean percentage increase of all the rats on any one treatment surviving at that time.

Over a period of 10 weeks the controls gained only some 4% in body weight (7 g.) while the free-fed growth-hormone-treated group gained 50%, representing an increase of 95 g. The limit-fed injected group grew to an intermediate degree, the total increment amounting to about 35% (60 g.). The

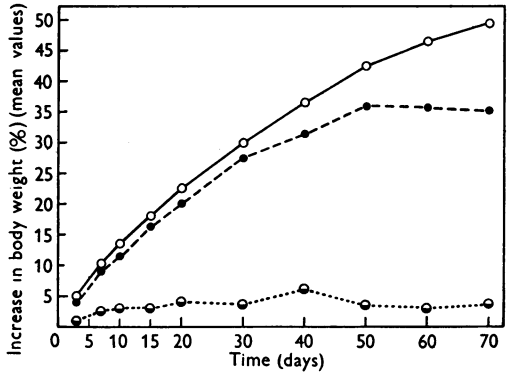

Fig. 1. Growth curves of normal rats (◒) and growth-hormone-treated rats (*a*) on unlimited food intake (○), and (*b*) limit-fed with the control rats (●).

relative rates of growth of the free-fed and limit-fed groups are of particular importance. The free-fed group increased in weight in almost linear fashion. Initially the limit-fed group grew at a rate only slightly less, but this had begun to decline by the 40th day and soon after, this group of rats practically ceased to grow, the curve reaching a plateau at about the 50th day. Over the entire period of time the controls grew but slightly and in an irregular fashion.

These changes in body weight are reflected in the protein content of the carcass of these rats (Fig. 2). The total protein of the control animals increased from an initial value of 32 g. to a final value of 40 g., an increase of only 25% in 70 days, whereas the free-fed injected group contained 65 g. of protein at the end of this period, an increase of 86% over the initial value of 35 g. The increase in the limit-fed group was intermediate, the total protein rising from 35 to 55 g., that is by 57%.

The changes in the fat content of the carcass are roughly the converse of the protein changes (Figs. 3 and 4). The picture is complicated by the striking increase in the fat levels between the 3rd and 15th days. Temperatures in the animal house were excessive; the day temperature exceeded 28° throughout this period and never fell below 16° during the night. At this ambient temperature the

rats were lethargic, as evidenced by the data collected on two control rats kept in cages mounted on a pivoted platform which rocked each time the rat crossed the mid-line of the cage. The number of

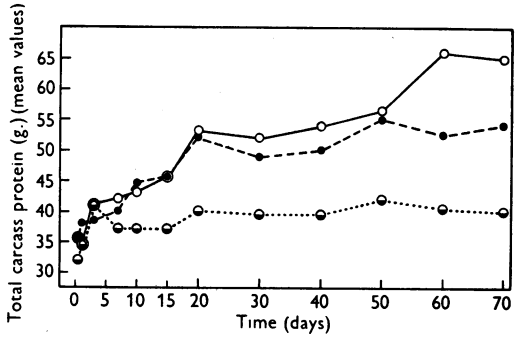

Fig. 2. Protein content of normal rats (◒) and growth-hormone-treated rats (*a*) on unlimited food intake (○), and (*b*) limit-fed with the control rats (●).

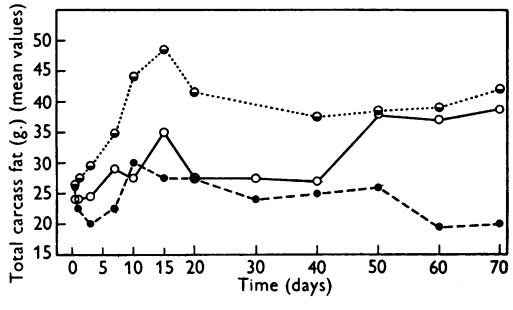

Fig. 3. Changes in the fat content of normal rats (◒) and growth-hormone-treated rats (*a*) on unlimited food intake (○), and (*b*) limit-fed with the control rats (●).

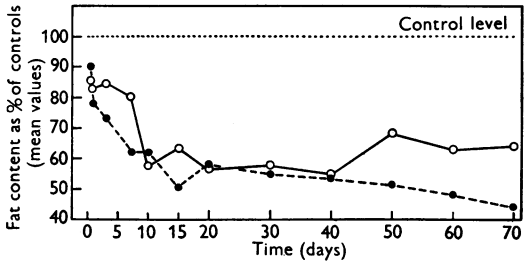

Fig. 4. Fat content of growth-hormone-treated rats (*a*) on unlimited diet (○), and (*b*) limit-fed with control rats (●) expressed as a percentage of the fat content of the control animals.

times the cage rocked was recorded on an automatic counting device. The two control rats made, on an average, only eighteen spontaneous movements during the 24 hr., instead of a mean of 103 movements a day recorded between the 1st and 3rd days and 20th and 30th days.

Since the rats continued to eat the same amount of food during the period of reduced activity, this was in excess of energy requirements, and as a consequence some of this excess was stored in the body as fat. Thus, over the period from the 3rd to the 15th day the control rats stored an additional 19 g. of fat in the body. The rats receiving unlimited diet also showed a reduction of spontaneous movement and in these animals, too, extra fat was stored in the body, although the increase was only 10 g. The limit-fed group stored least fat of all, only 7 g., although these animals were likewise affected by the heat and only moved 17–20 times during the 24 hr.

The remarkable increase in the fat content of the control group and the lesser increases of the two injected groups are of considerable interest in the light of the experiments of Ingle (1951), which showed that forced reduction of activity leads to excessive adiposity. It may be presumed that the increase in fat content of the rats described here, between the 5th and 15th days, was a direct consequence of the reduced activity. After this period, when the temperature and activity had returned to normal, the pattern of the fat changes became clearer. The amount of fat in the carcasses of the control rats remained unchanged over the next 50 days, and the level of fat in the free-fed group slowly increased almost to control levels. In the limit-fed group, however, the total body fat fell over the whole period until, after 70 days, the carcasses of these rats contained only half the fat found in the control group, in spite of the fact that the body weights of the injected rats were over 70 g. higher than those of the controls.

Fig. 4 shows the relative changes in the percentage fat present in the carcasses of these rats. The percentage of fat in the bodies of the two growth-hormone-treated groups are expressed as a percentage of that present in the control animals. This mode of expressing the results was chosen in order to eliminate variations of the final body weights, and of other components of the carcass. This figure clearly illustrates the changes in the fat content of both the limit-fed and free-fed groups relative to the control group. Both treated groups lost fat rapidly, so that by the 10th day they contained, relatively, only about 60 % of the control level. In the free-fed group the relative quantity of fat remained substantially unchanged during the rest of the experiment and even increased somewhat during the last 20 days. On the other hand, the relative fat content of the limit-fed group decreased progressively from the 10th day, so that by the 70th day this group contained only about half the proportionate amount of fat of the controls.

Respiratory exchanges of whole animals

In addition to the measurements of the changes in the chemical composition of the rats, the respiratory exchanges of intact animals, of isolated liver slices and of diaphragm from untreated and growth-hormone-treated rats were also studied.

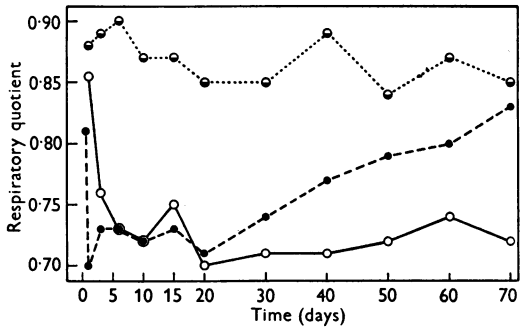

Fig. 5. Changes in the respiratory quotient of normal rats (◒) and of growth-hormone-treated rats (*a*) on unlimited food intake (○), and (*b*) limit-fed with the control rats (●).

Changes in the oxygen uptake and carbon dioxide output of normal and injected rats are shown in Table 1 and of the respiratory quotient in Fig. 5, in which each point, except the 70-day value, represents the mean value of three determinations. The oxygen consumption of the control rats did not vary over the whole experimental period of 70 days and this is reflected in the constancy of the figure for oxygen uptake per 100 g. of body weight. It is surprising that no decline in the oxygen consumption of the control rats was found between the 5th and 15th days when the animals were torpid due to heat prostration, and no explanation can be advanced to account for this result.

For the free-fed group the oxygen uptake appears to increase as the period of treatment lengthens, but nevertheless the increase fails to keep pace with the increment in body weight, and this is illustrated by the decline in oxygen consumption of animals in this group when expressed on the basis of oxygen uptake/100 g. of body weight (see also Fig. 5). The greatest oxygen uptake was found in general in the limit-fed group at most points in this experiment and, furthermore, it also showed a tendency to increase with increasing body weight. As with the free-fed group the body weight increased faster than the oxygen consumption, but because of the lower rate of somatic growth (Fig. 1) the discrepancy was not so great in the limit-fed group. The lowest value recorded in the limit-fed group was 46·9 ml. oxygen/100 g. of body weight as compared with 40·3 ml. oxygen/100 g. of body weight in the free-fed group.

More important were the changes in R.Q. As was to be expected the control rats had a R.Q. of about 0·88 and varied but very little from this figure. On the other hand, the free-fed rats began with a R.Q. of the same order as the control group, 0·86, but by the 3rd day of injections this had fallen to 0·76 and by the 6th day had declined to 0·73; this low level persisted throughout the remainder of the experiment. In the limit-fed group, however, the first measurement of R.Q., after 24 hr., showed the low value of 0·70. The R.Q. oscillated between 0·71 and 0·74 during the next 30 days and then rose to 0·77 on the 40th day of treatment. The rise in R.Q. continued until by the 70th day it had reached a value of 0·83, almost control level. The increase in the R.Q. was entirely attributable to the increase in carbon dioxide output.

Changes in the respiratory quotient of liver slices

Similar changes in the R.Q. were found when the tissues of rats, both normal and those treated with growth hormone, were investigated. The measurements were made in the presence of 0·02 M-glucose or acetate, or in the absence of any added substrates. Changes in the R.Q. of liver slices are shown in Fig. 6.

Table 1. *Respiratory exchange of control rats and rats injected with growth hormone and receiving restricted or unrestricted food*

No. of days of injection	O_2 uptake (ml.)			CO_2 output (ml.)			O_2 uptake/100 g. body weight (ml.)		
	Control	Free-fed	Limit-fed	Control	Free-fed	Limit-fed	Control	Free-fed	Limit-fed
1	108	104	117	95	90	82	51·7	51·3	59·1
3	117	107	122	104	81	89	56·5	50·1	59·3
6	104	116	109	94	85	80	49·4	51·5	50·5
10	116	112	116	101	81	84	54·2	47·9	52·3
15	112	118	123	97	89	90	51·9	48·2	52·8
20	113	121	114	96	85	81	52·0	47·6	47·5
30	120	124	120	102	88	89	55·3	47·0	47·6
40	118	130	126	105	92	97	53·7	46·1	47·0
50	110	128	133	92	92	105	49·8	43·0	48·5
60	111	129	138	96	94	111	50·2	40·3	51·5
70	106	130	131	90	94	109	47·1	41·1	46·9

No added substrate. The R.Q. of liver slices from the uninjected control group did not vary unduly and had a mean value of 0·86. In the free-fed group the first measurement, 12 hr. after the injection of growth hormone, had declined to 0·81 but after a further 12 hr. this value had fallen precipitously to 0·63. By the 3rd day the value had increased to 0·75 and after this time it remained remarkably constant around 0·76. Even more striking changes were obtained with liver slices from injected rats receiving limited diet. As soon as 12 hr. after the first injection, a value as low as 0·65 was reached and the R.Q. remained below 0·70 up to the 10th day. The R.Q. then rose to the same level as that of the free-fed group and remained so up to the 40th day. After this the R.Q. increased progressively until, by the 70th day, it had reached a value (0·86) clearly above the free-fed level and indistinguishable from the control values.

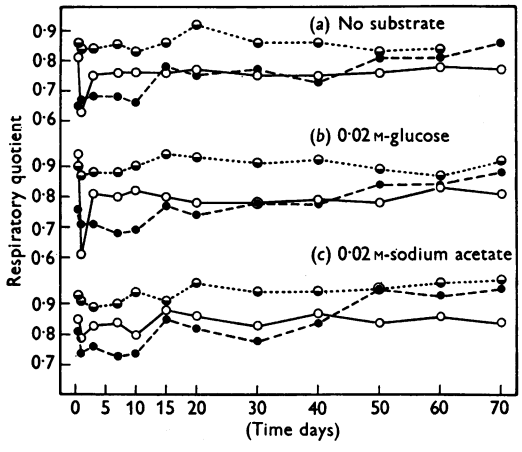

Fig. 6. Changes in the respiratory quotient of liver slices from normal rats (◒) and from growth-hormone-treated rats (i) on unlimited food intake (○) and (ii) limit-fed with the control rats(●). (*a*) In the absence of added substrate, (*b*) in the presence of 0·02 M-glucose, and (*c*) in the presence of 0·02 M-sodium acetate.

In the presence of 0·02 M-*glucose.* In the presence of 0·02 M-glucose substrate the R.Q. of liver slices from the control group was again very steady with a mean value of 0·90. This mean value is higher than that found in the absence of added substrate. The changes in R.Q. of liver slices from the free-fed group also resembled the corresponding values found in the absence of added substrate. The high value of 0·92 found in the free-fed group after 12 hr. treatment with growth hormone shows a lack of effect similar to that observed in the absence of substrate, and this parallelism is further underlined by the dramatic fall in the next 12 hr. when the R.Q. had declined to 0·61. Recovery was again complete by the 3rd day, after which the R.Q. was sensibly constant at a value of 0·80 and this, like the R.Q. of the uninjected group, was somewhat higher than the value obtained in the absence of substrate. The similarity between the R.Q.'s of liver slices using endogenous substrates and those with added glucose is further exemplified by the changes in the limit-fed group. A depression of the R.Q. was detectable as soon as 12 hr. after the beginning of growth-hormone injections, the value then being 0·76. Continued injections produced a further fall to 0·70 and between the 7th and 10th days to values below 0·70, but thereafter the level increased first to 0·77 from the 15th to 40th days, to 0·84 on the 50th and 60th days and to 0·88 on the 70th day. This latter value is well above those of the free-fed group and is in reasonable agreement with those of the control group.

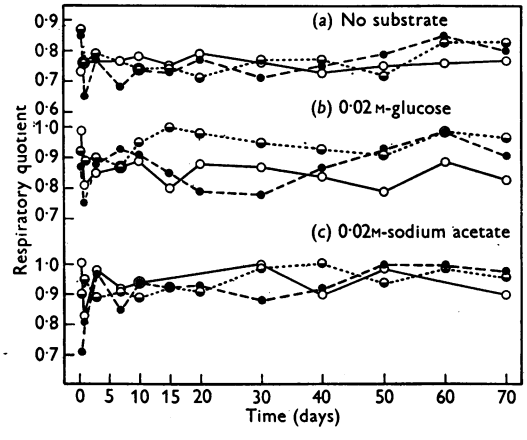

Fig. 7. Changes in the respiratory quotient of slips of diaphragm from normal rats (◒) and from growth-hormone-treated rats (i) on unlimited food intake (○) and (ii) limit-fed with the control rats (●). (*a*) In the absence of added substrate, (*b*) in the presence of 0·02 M-glucose, and (*c*) in the presence of 0·02 M-sodium acetate.

In the presence of 0·02 M-*sodium acetate.* The pattern of change in the presence of sodium acetate as substrate was much the same as that described above for glucose. The liver slices from uninjected controls had a relatively steady R.Q., averaging 0·94. The free-fed injected group had an R.Q. of 0·85 after 12 hr. and 0·79 after 24 hr. but thereafter rose to a value of 0·84 at which value it remained with only minor variations. The R.Q. of the injected rats receiving limited food was down to 0·81 in the first 12 hr. and 0·74 in 24 hr. It remained at this low level until the 10th day and then rose to an intermediate value around 0·80 until the 40th day. R.Q.'s of 0·95, 0·93 and 0·95 were recorded on the 50th, 60th and 70th days of injection.

It should be noted that in each of the three sets of curves shown in Fig. 5 the greatest R.Q. was always found in the control rats. In the free-fed injected rats there was an abrupt decline in the first 24 hr. but this had been partly reversed by the 3rd day. After this time the value remained substantially constant for the remaining 67 days of the injection period. The depression of the R.Q. was always earliest and most prolonged in the case of the limit-fed group, but both in the presence of added substrate and when the liver slices were metabolizing endogenous substrates, the low R.Q. always rose to the free-fed level around the 40th day and to the control level by the 70th day.

Respiratory quotient of diaphragm slips

Changes in the R.Q. of slips of diaphragm are shown in Fig. 7. These are very similar to those found in liver. No great reliance is placed on these changes since as the treated rats grew in weight their diaphragm increased in thickness and in the latter half of the experiment were certainly thicker than 0·5 mm.

DISCUSSION

The growth curves of the three groups of rats described above resemble those previously reported by Young (1945) in showing that while the growth of rats injected with pituitary growth hormone and given unlimited food is continuous and almost linear over the whole period of the experiment, rats similarly treated but on limited diet grow in a linear fashion for a period of only about 50 days.

The experiments described here strongly suggest that the decline in growth rate of the limit-fed injected group could not have arisen from the development of a resistance to the hormone, but rather show that while the two different levels of food intake both allow vigorous growth in the early stages of hormone treatment, continued treatment over a long period on limited diet leads to the exhaustion of the supply of some necessary endogenous material, so that further injections can no longer induce a response. Evidence is presented in this paper which is consistent with the view that the loss of response in limit-fed animals is due to the exhaustion of the labile fat depots in the growth-hormone-treated animals. Gaebler (1933) treated dogs receiving a constant amount of food with partially purified anterior pituitary extracts and noted a disappearance of the growth response after 3 months of continuous injection and a restoration of this response after the animals had rested for 2 months. The restoration of response after 2 months rest in these dogs was possibly due to a replenishment of fat depots during the resting period.

The results of the carcass analyses lend emphasis to this view point. The changes in carcass composition resulting from continued injections of pituitary-growth hormone confirm and extend the work of Young (1945) and of Li *et al.* (1948). The protein content of the bodies of rats both on unlimited and on constant diet increased progressively for 50 days. As was to be expected, the rate of the protein deposition in the tissues was greater in those animals receiving food *ad lib.* than in those on a limited food intake, and, further, in the former group the total protein content increased continuously throughout the 70 days of the experiment. However, in the latter group no further increment occurred after the 50th day.

As in the experiments of Young (1945) the difference in fat content between the control group and the limit-fed group is enough to account for the increment in protein content of the experimental animals, if it is calculated that each g. of fat catabolized allows the deposition, on an equicaloric basis, of 2 g. of protein and about 6 g. of attendant water. It should also be noted that the percentage fat content of the limit-fed group reached its lowest figure by the 60th day, that is coincidentally with the cessation of somatic growth and protein deposition.

These facts suggest a close correlation between the rate of new protein synthesis and fat dissimilation in the growth-hormone-treated animal on limited food intake, with protein synthesis stopping when no labile fat reserve remains to be catabolized. This state of affairs seems to be reached in the rat when the fat content of the body falls to around 7 %, a value which seems to be an irreducible minimum and which presumably represents those lipids (phospholipin, cholesterol, etc.) which constitute an integral part of the cell structure and are not available for energy purposes.

In this light, pituitary-growth hormone is presented as a hormone having a specific action on fat catabolism, although it is not suggested that this is its only action. Evidence is presented in the two succeeding papers (Greenbaum & McLean, 1953*a*, *b*) that growth hormone does indeed have a direct action on fat mobilization and catabolism, but in this paper the indirect evidence of R.Q. measurements, both of the intact animal and of its tissues *in vitro*, is presented to show that the period of growth induced by growth hormone is associated with a period of fat catabolism, and that changes of R.Q. indicative of a decrease in fat catabolism, are associated with a slowing and even cessation of growth.

Measurements of R.Q. in the intact animal are highly suspect, particularly when, as in the present case, there is an associated ketosis. Nevertheless, it is felt that the differences between the R.Q.'s of the normal untreated controls and the growth-hormone-treated groups reported here are sufficiently great

and consistent to warrant the interpretations placed upon them. Further, in the growth-hormone-treated rat the body is growing so rapidly that amino-acid catabolism is at a minimum, the R.Q. measured approaches the non-protein R.Q. and the interpretation is therefore made somewhat more reliable. The fact that the measurements were always made at the same time of day and at the same time in relation to the last feed also tends to increase the reliability of the measurements. Throughout this experiment the R.Q. of the untreated animal was about 0·88 with only insignificant variations from this figure, indicating that these rats were catabolizing a mixture of carbohydrate, fat and protein. The injection of growth hormone caused a prompt decrease in the R.Q. to 0·75 in the free-fed and 0·70 in the limit-fed rats indicative of a change to a catabolism almost exclusively based on fat. This low level was maintained by the free-fed groups throughout the period of injection and it should be noted that this group of animals maintained its rate of growth over the whole period of observation.

In the limit-fed group, on the other hand, the low R.Q. was maintained for only 20 days and thereafter began to rise. By the 50th day it had risen to 0·80 and later to a value indistinguishable from the control level. It should be noted that the rise in R.Q. to 0·80 and above, values consistent with the catabolism of metabolites other than fat, coincides with the cessation of growth.

Similar changes to those in the intact animal were found in the R.Q.'s of isolated tissues *in vitro* presumably because the tissues were saturated with growth hormone. In the hormone-treated groups a striking initial decline to values about 0·70 again lends support to the view that one of the earliest responses to growth hormone is a great increase in fat catabolism. This effect is shown by the tissues in the absence of added substrate as well as in the presence of added glucose or acetate. An interesting point is that during the first 10 days of treatment the R.Q. of liver slices from both injected groups falls below 0·7, the theoretical value for fat catabolism, even in the presence of glucose, and this indicates CO_2 retention. This may be associated with the formation, in the liver, of acetoacetate which is known to occur after treatment with pituitary-growth-promoting extracts (Bennett, Kreiss, Li & Evans, 1948). The observations here are also interesting in that they throw some light on the metabolic behaviour of muscle tissue after growth-hormone treatment. If the diaphragm were merely oxidizing acetoacetate produced in the liver this would give an R.Q. of 1·0, but in fact the value found is 0·7 and this may well indicate an extrahepatic utilization of fatty acids.

All the evidence presented in this communication is consistent with the view that growth in pituitary-growth-hormone-treated rats is dependent not only on a direct reduction of protein oxidation, but also on an increase in the catabolism of fat to provide the calories for energy and to spare dietary protein for deposition in the tissues.

In the animals with access to unlimited food, the extra food consumed and the energy derived therefrom permits continued growth of the rat over the whole period of the experiment. In the limit-fed group, however, no extra calories are obtained by an increased food intake, and as a consequence the fat depots of the body are oxidized until no depot fat remains. With the whittling away of the endogenous supply of calories all the dietary components must be oxidized to provide the energy for maintenance, and growth ceases. It seems likely that the role of fats in this system is to provide calories and not, as Li & Evans (1948) suggest, to provide carbon skeletons for protein synthesis. In this connexion it would be interesting to investigate whether an increase in the absolute quantity of carbohydrate fed after the fat reserves have been depleted, that is the provision of a fresh source of calories, would spare the dietary amino-acids and permit a resumption of growth. Growth hormone must therefore be regarded as a specific stimulator of fat catabolism.

SUMMARY

1. Two groups of rats, one receiving unlimited diet and the other limit-fed with a group of controls, were injected with a preparation of crystalline growth hormone. Both groups increased in weight, the free-fed group continuously throughout the period of 70 days during which they were injected and the limit-fed group at a lesser rate for 50 days after which growth practically ceased.

2. Carcass analyses throughout the 70 days showed a continuous increase in the protein content and a progressive decrease in the fat content of the treated animals compared with the controls.

3. The respiratory quotients of animals from each of the three groups of rats have been measured at intervals throughout the 70 days of the experiment. The respiratory quotient of the control group remained substantially constant at a value of 0·88. The respiratory quotient of the two injected groups fell to 0·70–0·75 within 24 hr. after the beginning of the injections and in the case of the free-fed animals remained at this low level for the duration of the experiment. The respiratory quotient of the limit-fed group remained at the low value for only 20 days and rose steadily thereafter to a value indistinguishable from the control levels by the 70th day.

4. Measurements of the respiratory quotient of slices of liver and slips of diaphragm showed

essentially the same picture; an initial depression of the respiratory quotient which was maintained in the case of the free-fed animals but which, in the case of the limit-fed group, rose to the control levels after 50 days of treatment.

5. The results are believed to demonstrate that protein deposition in growth-hormone-treated rats can only take place as a result of an increased emphasis on fat catabolism, with a consequent sparing of dietary proteins for deposition in the tissues. As a corollary to this, it has been shown that in limit-fed rats growth ceases when the body stores of labile fat are exhausted. It is suggested that one of the primary effects of growth hormone activity is in the catabolism of fat.

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The Mobilization of Lipid by Anterior Pituitary Growth Hormone

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Injections of growth-promoting anterior pituitary extracts into rats have been shown to increase the nitrogen and decrease the fat content of the carcass (Lee & Schaffer, 1934; Young, 1945). Similar effects have been demonstrated with purified growth hormone (Li, Simpson & Evans, 1948; Greenbaum, 1953). The importance of pituitary growth hormone in controlling fat metabolism has also been indicated by the lowering of the respiratory quotient (Gaebler, 1933*a*, *b*; Greaves, Freiberg & Jones, 1940; Greenbaum, 1953), and by the massive and rapid mobilization of fat to the liver found after injections of the hormone. Doses as low as 100 μg. of purified growth hormone have been shown by Weil & Ross (1949) to cause a significant increase in the liver fat of mice within 2 hr. after treatment and a doubling of the control level after 7 hr. Li, Simpson & Evans (1949) observed a similar increase when they examined the effect of acute treatment with growth hormone (5 mg. administered over a period of 6 hr.) in rats previously starved for 24 hr. In these animals the liver fat was increased by 65 % in 6 hr. These authors also reported that when the treatment was extended over periods longer than 10 days this effect was reversed, and the fat content of the liver was always decreased. They suggested that growth hormone first stimulated a mobilization of fat to the liver and subsequently increased its rate of oxidation.

Barrett, Best & Ridout (1938) and Stetten & Salcedo (1944) have used deuterium to label the depot fats of the body, and have shown from the rate at which deuterated fats accumulated in the liver after injections of growth-promoting pituitary extracts, that the primary source of this new lipid was the body depots. There have been relatively

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