SUMMARY

1. The coupling reaction between cyclomaltohexaose and isomaltose, catalysed by *Bacillus macerans* transglycosylase, was carried out and the products were degraded with β-amylase.

2. A trisaccharide, proved to be 6-α-maltosylglucose, was isolated as a β-amylase-resistant end product. A tetrasaccharide, believed to be 6-α-maltotriosylglucose, was also isolated. This was slowly hydrolysed by β-amylase.

3. The rate of hydrolysis of 6-α-maltosylglucose in hot aqueous acid is the same as of the isomeric trisaccharide panose (4-α-isomaltosylglucose).

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Plasma Glucose, Non-Esterified Fatty Acid and Insulin Concentrations in Hypothalamic-Hyperphagic Rats

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Hyperphagia may be induced in rats by electrolytic lesions in or near the ventromedial nuclei of the hypothalamus (Hetherington & Ranson, 1940), although the mechanism of the appetite disturbance is unknown. There is much evidence in favour of the suggestion by Mayer & Bates (1952) that food intake normally varies in inverse proportion to the peripheral arteriovenous difference in the concentration of glucose, which to a certain extent reflects the uptake of glucose by muscle, and it has been proposed that specialized 'glucoreceptors' provide the hypothalamic regulatory centres with information about this rate of uptake. If this highly specific signal to the hypothalamus provides the regulatory mechanism then other changes in metabolism can only affect appetite indirectly by modifying the rate of uptake of glucose (Mayer, 1955).

As rats with acute lesions of the hypothalamus become fat their intake of food gradually returns to normal (Kennedy, 1950, 1953), and our first object was to find out whether obesity modified carbohydrate metabolism in any way that might explain its inhibitory effect on appetite.

The metabolism of glucose and that of non-esterified fatty acids are interrelated in such a way that in the mammal either one may decrease the oxidation of the other (Randle, Garland, Hales &

Newsholme, 1963). Insulin acts with glucose to diminish the availability for oxidation of non-esterified fatty acids by decreasing the release of the non-esterified fatty acids from adipose tissue and by promoting the esterification of fatty acid in muscle. On the other hand, the effect of insulin in increasing glucose uptake by muscle may be decreased by an increased availability of non-esterified fatty acids for oxidation. Therefore, in assessing glucose metabolism in the whole animal, it is important to know the plasma concentrations of glucose, non-esterified fatty acids and insulin. In the present study these concentrations have been measured in rats at different times after the induction of hyperphagia by lesion of the hypothalamus in order to define more precisely the metabolic behaviour of this preparation.

MATERIALS AND METHODS

Animals. Adult female rats of the Lister hooded strain, weighing 180–220 g. and housed at 24°, were used. All animals were fed on the M.R.C. diet 41 B. Starved animals were deprived of food from 9 a.m. for a period of 24 hr. followed by anaesthetization and withdrawal of blood.

Induction of hyperphagia. Lesions were introduced into the region of the ventromedial nuclei of the hypothalamus stereotaxically as described by Kennedy & Mitra (1963).

Collection of plasma. Animals were anaesthetized by intraperitoneal injection of pentobarbitone sodium B.P. (60 mg./kg.). An abdominal incision was made and blood withdrawn from the abdominal aorta into a syringe containing 500 i.u. of heparin. Plasma was separated by centrifugation.

Determination of plasma glucose concentration. The glucose-oxidase method of Huggett & Nixon (1957) was used to determine the glucose content of a 0·1 ml. sample of plasma after deproteinization with zinc hydroxide (Somogyi, 1945).

Determination of plasma non-esterified fatty acid concentration. Samples (1 ml.) of plasma were extracted and titrated according to the method of Dole as modified by Trout, Estes & Friedberg (1960).

Determination of plasma insulin concentration. Duplicate 0·1 ml. samples of undiluted plasma were assayed by an immunoassay method described by Hales & Randle (1963*a*). The results are expressed in terms of equivalent concentrations of standard solutions of ox insulin (6 × recrystallized; stated potency 22·3 i.u./mg.). A comparison of the standard curve of pure ox insulin with that of pure rat insulin is shown in Fig. 1. In the assay system employed the two curves are not parallel and there is a greater decrease in recovery of radioactivity produced by standard ox insulin than by rat insulin at equal concentrations over the range tested. Rat pancreatic insulin consists of two molecular species (L. F. Smith; quoted by Young, 1961). The composition of rat plasma insulin is not known and therefore it is not possible to express the rat plasma insulin concentrations in terms of pure rat insulin. Since the depression of recovery of radioactive insulin produced in the assay by ox insulin and rat insulin differs over the plasma range of insulin concentrations and since the two curves are not parallel, the assay will underestimate the true concentration of rat insulin and also any differences in concentration observed. Nevertheless, as there is an increasing loss of radioactivity from the insulin–(insulin antibody) complex produced by increasing concentrations of pure rat insulin it is possible to assay rat plasma insulin and express the results on an arbitrary scale (in this case equivalent amounts of pure ox insulin). If a significant difference in insulin concentrations between two groups of rats is demonstrated then the above considerations mean that the real difference will be even larger than that estimated.

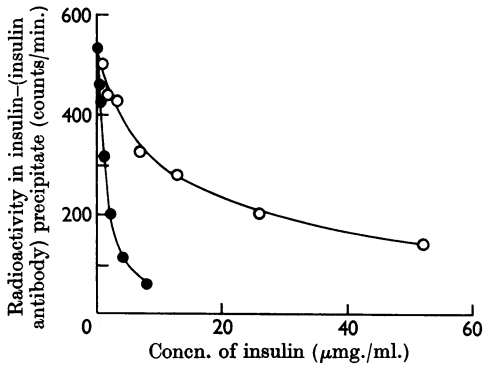

Fig. 1. Effect of unlabelled ox insulin (●) and rat insulin (○) on the recovery of ^{131}I-labelled insulin in insulin–(insulin antibody) precipitate. Details are given in the text.

RESULTS

Acute effect of lesion. To determine whether the induction of hypothalamic lesions leading to hyperphagia led to alteration of plasma glucose, non-esterified fatty acid or insulin concentrations independent of the chronic ingestion of increased amounts of food, hypothalamic lesions were made in a number of rats which were then allowed unlimited access to food for 24 hr. after the operation. On the basis of the food taken over this period two groups each of five rats were selected, one having normal (15–20 g.) and the other a raised (35–40 g.) daily intake of food. The two groups were starved for 24 hr. and then bled. No significant difference in the starvation concentrations of plasma glucose, non-esterified fatty acid and insulin were detected (see Table 1*a*).

Effect of lesion plus 7 days of hyperphagia. The effect of 1 week of hyperphagia was investigated (Table 1*b*). Hyperphagic animals were allowed unlimited food for 1 week. One group was then bled and another group starved for 24 hr. before being bled. The fed hyperphagic animals had a lower plasma glucose concentration than the controls although this difference did not reach statistical significance at the 5% level. The plasma non-esterified fatty acid and insulin concentrations

Table 1. *Plasma glucose, insulin and non-esterified fatty acid concentrations in rats with hypothalamic lesions and in control rats*

(*a*) Acute effect of lesion: the animals with hypothalamic lesions were fed *ad lib.* for 24 hr. after the operation, then starved for 24 hr.; the control animals were treated similarly, but did not prove to be hyperphagic. (*b*) Effect of lesion plus 7 days of hyperphagia: hypothalamic-hyperphagic animals were fed *ad lib.* for 7 days and then either bled immediately or starved for 24 hr. before being bled; the control animals were of the same age and sex but had not received hypothalamic lesions. (*c*) Effect of lesion plus 3 months of hyperphagia: hypothalamic-hyperphagic animals were fed *ad lib.* for 3 months and then either bled immediately or starved for 24 hr. before being bled; the control animals were of the same age and sex but had not received hypothalamic lesions. Details are given in the text. The concentrations of insulin are expressed as equivalent amounts of ox-insulin standard, all samples being assayed undiluted.

	No. of rats	Concn. of glucose (mg./100 ml.)	$10^6 \times$ Concn. of insulin (i.u./ml.)	Concn. of non-esterified fatty acids (μM)
(*a*) Acute effect of lesion				
Hyperphagic rats	5	119±6·3	28±4·2	350±26
Control (non-hyperphagic rats)	5	124±2·2	36±11	400±32
(*b*) Effect of lesion plus 7 days of hyperphagia				
Hyperphagic rats	6	126±3·5	106±7	170±15
Control rats	5	140±5·2	61±6·3	70±16
Hyperphagic rats (starved for 24 hr.)	6	103±3·3	53±2·5	650±75
Control rats (starved for 24 hr.)	5	110±5·0	33±3·7	680±59
(*c*) Effect of lesion plus 3 months of hyperphagia				
Hyperphagic rats	5	160±29	191±59	470±25
Control rats	5	114±4·0	44±3·9	380±26
Hyperphagic rats (starved for 24 hr.)	5	110±6·0	74±8·0	1100±92

were elevated, both differences being significant ($P<0·01$).

There was no difference between the plasma concentrations of glucose and of non-esterified fatty acids of the starved hyperphagic and control animals, but the starvation plasma insulin concentration was significantly elevated ($P<0·01$) in the hyperphagic group.

Effect of lesion plus 3 months of hyperphagia. Two further groups of hypothalamic-hyperphagic animals were investigated after 3 months of unlimited access to food (Table 1*c*). All the animals were very obese (mean weight 520 g.; max. normal weight 250 g.). One group was fed and the other starved for 24 hr. before being bled. The fed hyperphagic group had higher concentrations of glucose, non-esterified fatty acids and insulin than fed controls of the same age. The increases of insulin and non-esterified fatty acid concentrations were significant at the 5% level. The starved hyperphagic rats showed no change in plasma glucose concentration. The tendency for the starvation insulin concentration to remain elevated was even more marked in these animals and there was now also a great increase in plasma non-esterified fatty acid concentration as compared with the starved animal after 7 days of hyperphagia ($P<0·05$).

DISCUSSION

Regulation of appetite. The metabolic abnormalities described above of hyperphagic rats are not present in animals with acute hypothalamic lesions but deprived of food and are, therefore, caused by overeating and not directly by the hypothalamic damage. The converse hypothesis, that an induced abnormality of, say, insulin secretion leads to a compensatory hyperphagia, is therefore untenable.

During early hyperphagia there was a high insulin output combined with a good response to insulin as judged by plasma glucose and non-esterified fatty acid concentrations; this would agree with Mayer's 'glucostatic' theory (Mayer, 1955; Mayer & Bates, 1952) as these changes would normally inhibit appetite and presumably failed to do so in rats with hypothalamic lesions because the glucoreceptors were damaged. The findings after 3 months of hyperphagia (a raised plasma glucose concentration in the presence of a very high plasma insulin concentration), together with reports of decreased glucose tolerance and delayed and diminished response to exogenous insulin in these rats (Brobeck, Tepperman & Long, 1943), are all consistent with the insulin resistance of muscle tissue *in vitro* shown by Randle *et al.* (1963) to result from increased availability of non-esterified fatty acids for oxidation. If increased oxidation of glucose were the sole satiety signal to the hypothalamus, the signal would appear to become weaker in the obese animal, and hyperphagia ought to be a self-accelerating rather than a self-limiting process. Insofar as adiposity inhibits appetite, therefore, it must do so in some way other than through the mechanism proposed by the 'glucoreceptor' theory.

Metabolic effects. The finding of a low plasma glucose concentration after 7 days of hyperphagia followed by a raised non-starvation plasma glucose

concentration after 3 months of hyperphagia confirms previous results (Mayer, Bates & Van Itallie, 1952; Brooks, 1946). The abnormalities in the chronic obese rat resemble those in human obesity in which raised concentrations of plasma non-esterified fatty acids (Dole, 1956) and insulin (Rabinowitz & Zierler, 1962) have been reported. Both conditions parallel the situation in the maturity-onset diabetic (Hales & Randle, 1963*b*) and these authors argued that the latter develops a resistance to the action of insulin in suppressing the release of non-esterified fatty acids by adipose tissue; the same defect could be postulated in the hypothalamic-obese rat.

The resistance to endogenous insulin of obese subjects may therefore be the result of an increased availability of fatty acid for oxidation. Dole (1956) found an increase in the starvation plasma non-esterified fatty acid concentration with increasing obesity. If the correction of obesity leads to a fall in the starvation plasma non-esterified fatty acid concentration then it might also be expected to lead to an improvement of carbohydrate tolerance. There is some disagreement as to whether this occurs (Aldersberg, 1958). However, any effect of the decrease of obesity on carbohydrate tolerance is likely to be dependent on the manner by which the restriction of calorie intake is achieved. A great decrease in carbohydrate intake is unlikely to improve carbohydrate tolerance, since a low-carbohydrate diet leads to an elevation of the starvation plasma non-esterified fatty acid concentration with insensitivity to endogenous insulin (Hales & Randle, 1963*b*).

The raised plasma insulin concentration in the early hyperphagic animal in the presence of a plasma glucose concentration that was lower than that of the controls requires an explanation. The hyperphagic animal eats for longer periods than normal and the gut rarely empties of food during the phase in which the animal is rapidly gaining weight. Consequently the plasma glucose concentration, although lower than that of the fed control, probably rarely falls to the starvation level unless food is withdrawn. The pancreatic β-cell is therefore exposed to prolonged stimulation and adaptive changes in its metabolism may well ensue, such that a greater insulin secretion is produced by a given concentration of glucose. Viñuela, Salas & Sols (1963) have shown that adaptive changes in hepatic glucokinase occur. Conversely, Hales & Randle (1963*b*) have shown that, in the situation of carbohydrate restriction, a relatively poor insulin response to a given glucose concentration results. A change in glucose sensitivity of the β-cells would also explain the insulin concentrations found in chronic obese rats. When these were fed their plasma glucose concentrations were high enough to account for their raised insulin output, but when they were starved the plasma glucose concentrations fell to the same as that of the fed controls; the insulin concentration, however, remained abnormally high.

SUMMARY

1. The induction of lesions of the ventromedial nuclei of the hypothalamus in rats led to the development of hyperphagia. Treated animals allowed unlimited access to food for 24 hr. followed by 24 hr. of starvation showed no detectable difference in the concentrations of plasma glucose, non-esterified fatty acid and insulin in comparison with similarly treated sham-operated controls.

2. Hyperphagic rats allowed unlimited access to food for 7 days were compared with untreated animals of the same age and sex and showed: (*a*) in the fed state significant increases of the plasma concentrations of non-esterified fatty acid and insulin; the plasma glucose concentration was lower than that of the controls but this difference was not statistically significant; (*b*) after starvation for 24 hr. a significant increase of the plasma insulin concentration with no change in the plasma concentrations of glucose and non-esterified fatty acid.

3. Hyperphagic rats allowed unlimited access to food for 3 months were compared with untreated animals of the same age and sex and showed: (*a*) in the fed state significantly higher plasma concentrations of non-esterified fatty acid and insulin; (*b*) after starvation for 24 hr. a persistent increase of the plasma concentrations of non-esterified fatty acid and insulin.

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The Effect of Chlortetracycline on the Transfer of Leucine and 'Transfer' Ribonucleic Acid to Rat-Liver Ribosomes *in vitro*

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Chlortetracycline prevents the passage of leucine from 'transfer' RNA to ribosomal protein in cell-free preparations from *Escherichia coli* and rat liver; the leucine is largely retained on the 'transfer' RNA (Franklin, 1963). Since attachment of 'transfer' RNA to ribosomal RNA may occur independently of amino acid incorporation into protein (Bloemendal & Bosch, 1962), it was decided to determine whether chlortetracycline prevents attachment of the amino acyl-'transfer' RNA to the ribosome or whether it prevents the subsequent condensation of amino acids into polypeptides. The present paper describes experiments with rat-liver preparations.

METHODS

Radioactive chemicals. Generally labelled L-leucine (L-[G-^{14}C]leucine) (24·8 mc/m-mole) and [6-^{14}C]orotic acid (11·6 mc/m-mole) were obtained from The Radiochemical Centre, Amersham, Bucks.

Chemicals. The sodium salt of ATP, the silver–barium salt of phosphoenolpyruvic acid, pyruvate kinase and pancreatic ribonuclease were obtained from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. The sodium salt of GTP was obtained from Pabst Laboratories, Milwaukee, Wis., U.S.A., and chlortetracycline hydrochloride from Lederle Laboratories Division, Cyanamid of Great Britain Ltd., London. Sodium dodecyl sulphate was obtained from L. Light and Co., Colnbrook, Bucks.

Preparation of 'transfer' ribonucleic acid labelled with [^{14}C]uracil and [^{14}C]cytosine. Each of four male adult white rats of the Laboratory Wistar strain received [^{14}C]orotic acid (0·43 μmole; 5 μc) dissolved in 0·9% sodium chloride solution (0·25 ml.) by intraperitoneal injection. The animals were killed 20 hr. later and the livers removed into an ice-cold medium (medium A) containing (final concentrations) tris–HCl buffer, pH 7·7 (40 mM), sucrose (0·35 M), $MgCl_2$ (10 mM), KCl (25 mM) and NaCl (75 mM). The pH 5 fraction (Franklin, 1963) was dissolved in water, and after the addition of sodium dodecyl sulphate (2 mg./ml.) the RNA was extracted by the method of Nirenberg & Matthaei (1961). Low-molecular-weight contaminants were removed by passage through Sephadex G-50 (Pharmacia, Uppsala, Sweden) (Bosch, Wende, Sluyser & Bloemendal, 1961). The specific radioactivity of the purified RNA was usually about 17000 counts/min./mg.

Preparation of 'transfer' ribonucleic acid labelled with [^{14}C]leucine. Rat-liver pH 5 preparations were labelled with [^{14}C]leucine (Franklin, 1963). The RNA was extracted and purified on Sephadex G-50 as described above except that the column was eluted with 1 mM-acetic acid instead of water.

Preparation of ribosomal ribonucleic acid. Ribosomes isolated after incubation as described below were resuspended in medium A and treated in the following different ways: (*a*) RNA was extracted by the method of Hoagland, Stephenson, Scott, Hecht & Zamecnik (1958) (single extraction with phenol); (*b*) RNA was extracted by the method of Nirenberg & Matthaei (1961) except that the preparation was not dialysed (triple extraction with phenol); (*c*) the ribosomes were twice extracted with 10% (w/v) sodium chloride at 100° after the washing procedure of Hoagland *et al.* (1958). The RNA was twice precipitated with ethanol.

Preparation of microsomes and ribosomes. Microsomes were prepared as described by Franklin (1963). To prepare ribosomes, the supernatant obtained after centrifuging the liver homogenate at 15000*g* for 15 min. was treated with $\frac{1}{9}$ vol. of 5% (w/v) sodium deoxycholate in 0·2 M-glycyl-glycine–NaOH buffer, pH 8·2, and centrifuged at 105000*g* for 90 min. The ribosomal pellet was resuspended in medium A (11 ml.) and centrifuged at 105000*g* for 90 min. Finally microsomes and ribosomes from 25 g. of liver were resuspended in medium A (10 ml.). In ribosomal preparations a small portion of the 15000*g* supernatant, not treated with sodium deoxycholate, was centrifuged at 105000*g* for 90 min. to obtain the final supernatant (105000*g* supernatant).

Conditions of incubation. A portion (2 ml.) of the particulate suspension (containing about 6 mg. of RNA)