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HYPOTHALAMIC (PHOTOPERIODIC) CONTROL OF A SEASONAL ANTAGONISM TO INSULIN IN THE RAT HEART

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The experiments of Rowan (1925) were the first to emphasize the primary importance of day length amongst environmental factors in the control of endocrine function in birds and mammals, and the appreciation of this fact has led to the generally accepted theory of the control of the anterior pituitary by neurosecretions from the hypothalamus (Harris, 1960). Of the seasonal changes in metabolism, the increased deposition of fat in winter in mammals and that before migration in birds have been shown to be photoperiodic effects (Hammond, 1954; Farner, 1961), and it is probable that the hypothalamic–pituitary axis is the regulating influence, although the hormonal agents involved are entirely unknown. As the presence of insulin is necessary for the formation and storage of fat, a possible regulating mechanism could involve modification of insulin effectiveness by a pituitary secretion, and in fact hypophysectomy and certain hypothalamic lesions result in insulin hypersensitivity (de Bodo & Altszuler, 1958). However, the mechanism cannot be merely an increased insulin sensitivity, for studies on three hereditary obesities in mice (Christophe, 1963), and those on obese human subjects (Karam, Grodsky & Forsham, 1963), reveal a decreased rather than an increased sensitivity to insulin. As insulin increases the glucose uptake of both skeletal muscle and adipose tissue, the mechanism could act by decreasing the insulin sensitivity of the proportionately larger component, muscle, without altering the action on adipose tissue. Consequently the effect of insulin on the latter tissue would appear enhanced as glucose was diverted from metabolism in muscle to lipogenesis in fat tissues.

Pituitary-dependent serum antagonists of insulin action on the glucose uptake of rat diaphragm have been described for several species (Krahl, 1961). Such a pituitary antagonist could constitute a physiological mechanism for the control of fat deposition by providing a restriction on

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the carbohydrate metabolism of striated muscle, thereby increasing the availability of glucose for lipogenesis. In the following account a fall in the insulin sensitivity of the isolated perfused rat-heart preparation during the winter months is described, which is further shown to be due to decreasing day length. The change in insulin response takes the form of a competitive inhibition of insulin action, and could be evidence of the seasonal participation of a normal antagonistic mechanism.

Fisher & Lindsay (1956) working with the isolated perfused rat heart showed that galactose, a sugar not metabolized by this tissue, is transported into the cells by an insulin-sensitive carrier mechanism which, furthermore, was shown to be the same as that responsible for the transport of glucose. The rate of galactose transport in the presence of insulin is too great to allow an accurate time course of penetration to be determined, but it is possible to follow the efflux from the heart previously loaded with the sugar by collecting coronary effluent in short, timed periods using a galactose-free perfusate. With this technique the differential effect of various insulin concentrations is readily observed. Preliminary accounts of some of this work have already been published (Fisher & Young, 1961; Young, 1962).

METHODS

Two groups of animals were used. At Oxford male albino rats of Wistar stock weighing between 200–300 g were used. These rats were kept in natural lighting conditions throughout the year, in conditions of constant temperature (22° C) for at least three days before use, and fed on stock laboratory diet (Styles Mineralized Rations, diet no. 41). The high carbohydrate diet used in some experiments with the albino rats was prepared by mixing 100 parts by wt. of a mixture containing 70% sucrose, 20% casein, 5% cod liver oil, 5% McCollum's salt mixture no. 185 (McCollum, Simmonds & Becker, 1922) with 72·5 parts of water and 25 parts of dried yeast extract. At Aberdeen, male hooded Lister rats were used, at weights ranging from 200 to 350 g. These rats were kept from birth in conditions of constant day length (12 hr) of artificial light and temperature (21° C). The change from use of albino rats kept on natural day length of 12 hr is specifically indicated in the text.

Perfusion technique. The procedure of Bleehen & Fisher (1954) was used except that a sintered glass filter (Zachariah, 1961) was substituted for the cellulose extraction thimble. When urethane was used as the anaesthetic, it was injected subcutaneously (5 g/kg). Hearts were perfused for 20 min with Krebs's-bicarbonate medium containing 55·6 mM galactose (1 g/100 ml.), washed out with a galactose-free perfusate and the effluent collected in timed samples. At the end of the experiment the heart was removed from the cannula, transected, blotted and homogenized. The homogenate was deproteinized with cadmium hydroxide (Fujita & Iwatake, 1931), excess cadmium ions removed by addition of barium carbonate (Miller & van Slyke, 1936) and filtered. The samples of coronary effluent and the heart extract were analysed for galactose by the copper reduction method of Nelson (1944).

Insulin. Two insulin preparations were used. Crystalline beef insulin 23 i.u./mg, part of a batch prepared by British Drug Houses Ltd. on behalf of the British Insulin Manufacturers (November 1955), was generously donated and was used throughout this work. The results with this preparation have been compared from time to time with those obtained with the commercial preparation 40 i.u./ml. (Burroughs Wellcome and Co., Ltd.) and no differences found. When insulin was used it was added to both perfusates at the same concentration.

Examination of the heart for other reducing substances

Concentrates of both the heart extracts and effluent samples in the presence and absence of both galactose and insulin were examined by paper chromatography for sugars and related reducing substances. Owing to the salt content the neutral solvent propanol:ethyl acetate:water (7:1:2) was used (Baar & Bull, 1953). The modified ammoniacal silver nitrate reagent (Trevelyan, Procter & Harrison, 1950) and the more specific aniline hydrogen phthalate reagent were used as location reagents. As observed with the rat diaphragm incubated in the presence of galactose (Resnick & Hechter, 1957), no reducing sugars other than galactose were found in any of the samples. The maximum limit of reducing substances which could have passed undetected was 3·0 % of the total galactose involved.

The result of a group of observations is presented as the mean ± S.E. of the mean (number of observations).

RESULTS

The time course of efflux from the heart

Hearts previously loaded with galactose as described in the section on methods were transferred to a galactose-free perfusate and samples of heart effluent collected in successive periods. After 7 min the hearts were removed from the cannulae and analysed together with the samples of effluent for galactose. The plot of the logarithm of the heart galactose content against time of efflux is shown in Fig. 1 for individual hearts

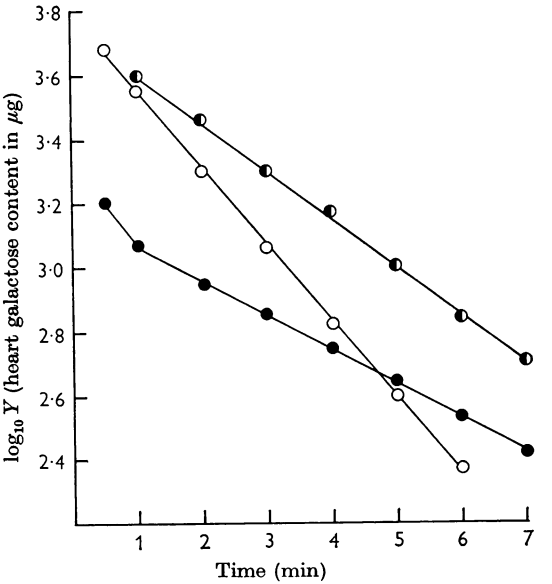

Fig. 1. The time course of the efflux of galactose from isolated hearts perfused with or without insulin: ● no insulin; ◐ 0·5 m-u./ml; ○ 3·0 m-u./ml. The logarithm of the heart content of galactose is plotted against time of wash-out with galactose-free medium. The curves show data for individual hearts which had been perfused with galactose (1 g/100 ml.) for 20 min. Insulin if present was included in both perfusates at the same concentration.

perfused with or without insulin. It was found that the wash-out of galactose from the heart was exponential from the end of the first minute for hearts perfused either with various concentrations of insulin or without the hormone.

In these circumstances an adequate means of expressing the rate of efflux is the fractional fall in heart galactose content during a particular time interval. The interval arbitrarily chosen was the fourth minute after transfer to the wash-out circuit, and the fractional fall is the fraction of the heart galactose content at 3 min that was removed in the subsequent minute.

Galactose efflux in the absence of insulin

The rate of galactose efflux was determined in hearts perfused with media without added insulin, and it was found that with ether anaesthesia the fractional rate of efflux was $0{\cdot}090 \pm 0{\cdot}011$ (10) in winter, and $0{\cdot}101 \pm 0{\cdot}022$ (4) in summer. Because of the very considerable variability of these results, urethane was used as an alternative anaesthetic to ether. This resulted not only in a reduction in variability, but also revealed a seasonal change in the fractional rate efflux of the sugar: in winter, $0{\cdot}050 \pm 0{\cdot}003$ (12); and in summer, $0{\cdot}094 \pm 0{\cdot}004$ (5). Decapitation, instead of anaesthesia, gave in winter fractional rates of efflux similar to those obtained with urethane: $0{\cdot}048 \pm 0{\cdot}006$ (4).

Insulin and galactose efflux

When insulin was included in both perfusates the rate of galactose efflux was increased; the change in the rate with increasing insulin concentration is shown in Fig. 2. As with hearts perfused in the absence of insulin, there was clearly a difference in the results between summer and winter. At higher insulin concentrations than can be conveniently included in Fig. 2, the curve for winter approaches that for summer, indicating the same maximal rate of efflux irrespective of the season. Thus at 3·0 m-u./ml. of insulin the fractional rates are $0{\cdot}409 \pm 0{\cdot}007$ (7) and $0{\cdot}363 \pm 0{\cdot}008$ (3) for summer and winter respectively; at 10 m-u./ml. the winter value has risen to $0{\cdot}380 \pm 0{\cdot}004$ (4). The same response to insulin was obtained with urethane anaesthesia as with ether.

Changes in insulin sensitivity throughout the year

The change in the response of the heart to insulin throughout the year is shown in Fig. 3. The period shown starts in July and ends in December of the following year. Two insulin concentrations (0·5 and 1·0 m-u./ml.) are considered.

The summer and winter insulin dose–response curves shown in Fig. 2 were obtained in September and January, respectively. A second dose–

response curve for summer, which was almost identical to that shown in Fig. 2, was obtained in the following September. The abrupt fall in insulin sensitivity which occurred during the autumn in both years took place during the second half of October, as the values quoted for this month were on both occasions obtained before 10 October.

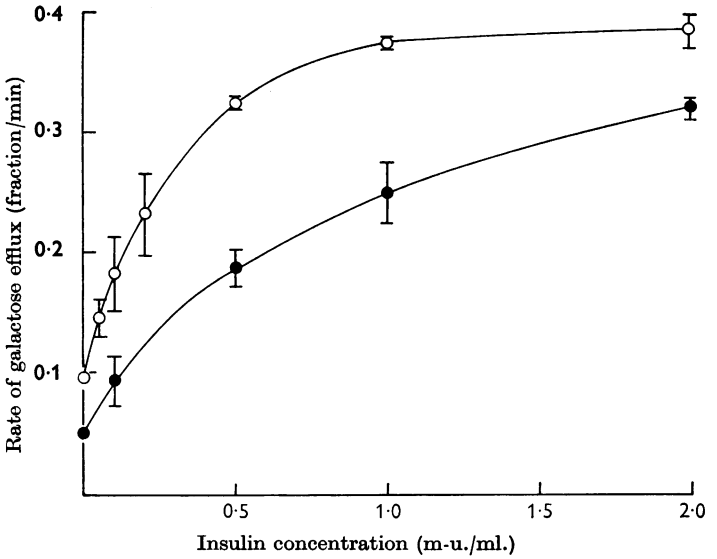

Fig. 2. The dose–response curves for insulin in summer ○ and winter ●. The response of the heart is measured as the fractional rate of efflux of galactose. The vertical bars represent ± S.E. of the mean of 4–8 measurements.

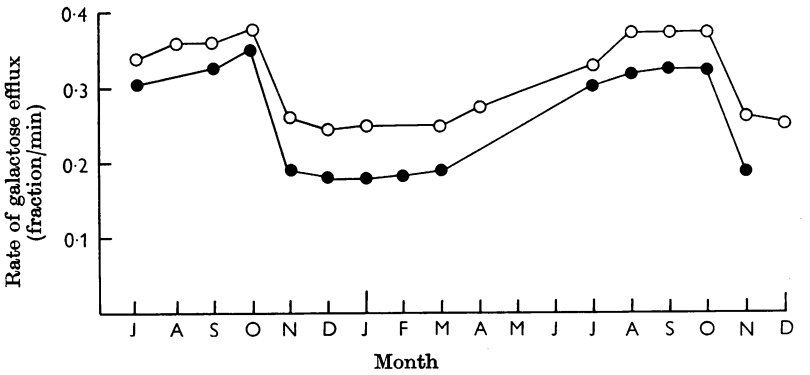

Fig. 3. The change in the response of the heart to insulin over the course of 1½ years, beginning in July and ending in December. The response, which is measured as the fractional rate of efflux of galactose, is recorded for two concentrations of insulin: ● 0·5 m-u./ml.; ○ 1·0 m-u./ml.

Effect of high carbohydrate diet

It was thought that the marked reduction in the response of the heart to insulin during October might be reversed by feeding the rats with a high carbohydrate diet, which according to Himsworth (1934) leads in rabbits to an increased sensitivity to insulin. Accordingly the following experiment was carried out in December. The animals were fed on the diet described in the methods section for 6 days before being killed and the rate of galactose efflux from the heart in the presence of insulin (1 m-u./ml.) was compared with that of hearts from rats fed on stock diet. The fractional rates were $0{\cdot}249 \pm 0{\cdot}020$ (12) for the experimental diet, and $0{\cdot}252 \pm 0{\cdot}019$ (12) for the stock diet. The corresponding value in summer on the stock diet was $0{\cdot}375 \pm 0{\cdot}004$ (8) (see Fig. 2).

Effect of change in day length

The hooded Lister rats used in the following experiments were kept throughout the year on a constant day length of 12 hr. Hearts removed from these animals in winter and perfused with media containing 1 m-u./ml. of insulin gave a fractional rate of efflux of $0{\cdot}363 \pm 0{\cdot}017$ (5), a value which was only obtained in summer with the albino rats maintained under natural lighting conditions ($0{\cdot}375 \pm 0{\cdot}004$ (8)).

A group of rats weighing between 80 and 100 g, which had been maintained since birth on a 12 hr day, was randomly divided into two groups. The control group continued on the constant day length of 12 hr. The day length of the other group, the experimental animals, was shortened by daily decrements for 6 weeks so that the natural daily decrement in day length corresponding to that of 26 September to 15 November (lat. 52° N.) was closely simulated. At the end of this period the response of the hearts to insulin (0·5 m-u./ml.), using galactose efflux, was determined for both groups. The fractional rate of efflux for the experimental animals was $0{\cdot}209 \pm 0{\cdot}022$ (6), whereas for the controls it was $0{\cdot}312 \pm 0{\cdot}023$ (6) ($P < 0{\cdot}01$).

The experiment was repeated with a constant daily decrement of 5 min over the first 40 days, and the experimental group was sampled at various intervals during the course of the experiment. There was a steady decrease in the response of the hearts to insulin (0·5 m-u./ml.) during the course of the experiment which was confirmed at its termination when the rate of galactose efflux was determined at three insulin concentrations (0·5, 0·25 and 0·125 m-u./ml.) (Table 1). The difference between the fractional rates of efflux of the control and the experimental groups at 48 days with 0·5 m-u./ml. of insulin was highly significant ($P = 0{\cdot}005$); at 30 days the difference was less significant ($P = 0{\cdot}05$).

TABLE 1. Effect of gradual reduction in day length on the response of the rat heart to insulin. The response is measured as the rate of galactose efflux

Duration of expt. (days)	Total reduction in day length (min)	Insulin concn. (m-u./ml.)	Rate of efflux (fraction/min)
Nil (controls)	Nil	0·5	0·345 ± 0·020 (7)
20	100	0·5	0·296 ± 0·008 (4)
30	150	0·5	0·275 ± 0·020 (5)
40 (controls)	Nil	0·5	0·342 ± 0·017 (6)
—	210	0·5	0·226 ± 0·028 (6)
—	—	0·25	0·186 ± 0·025 (4)
—	—	0·125	0·140 ± 0·008 (5)

TABLE 2. Effect of various constant day lengths on the response of the heart to insulin (0·5 m-u./ml.)

Day length (hr)	Rate of efflux of galactose (fraction/min)
12	0·344 ± 0·017 (6)
15	0·343 ± 0·010 (6)
18	0·338 ± 0·017 (8)
8½	0·318 ± 0·024 (8)

Effect of various day lengths of constant duration

The effect of a single abrupt change in day length from 12 hr to one of longer or shorter duration was investigated. Rats were kept on the new constant day length for 4 weeks, and the response of the heart to insulin (0·5 m-u./ml.) determined at the end of that time. The results are given in Table 2.

The effect of fasting

Albino rats were fasted for 12–15 hr before killing, in an attempt to obtain more reproducible conditions. There was no effect of such a period of fasting on the response of the tissue to insulin (1·0 m-u./ml.); the fractional rates of galactose efflux were: 0·371 ± 0·018 (4); and for fed animals, 0·373 ± 0·020 (4).

When hooded Lister rats were fasted for 24 hr, however, a very considerable reduction in insulin sensitivity was obtained. With 0·5 m-u./ml. of insulin, the fractional rate of efflux for the fasted animals was 0·227 ± 0·034 (5), and for the fed, 0·345 ± 0·013 (6). Rats fasted for 40 hr did not show any further decrease in insulin sensitivity; and, moreover, it was evident from the use of two insulin concentrations that, as with the photoperiodic inhibition already described, the inhibition due to fasting was reversible. At 0·5 m-u./ml. of insulin, the fractional rate of efflux was 0·249 ± 0·027 (5); and at 0·25 m-u./ml., 0·178 ± 0·005 (4).

DISCUSSION

Fisher & Lindsay (1956) and Park, Morgan, Henderson, Regen, Cadenas & Post (1961) have established that the transport of several hexoses and pentoses into the cells of the isolated perfused rat heart is achieved by a carrier mechanism, the activity of which is increased by insulin. The fact that galactose is not metabolized by the rat heart allows the study of the outward transport of this sugar from the cells; this transport is particularly sensitive to the action of insulin as the continuous removal of the sugar from the extracellular space reduces to a minimum transport back into the cell. That the wash-out of the sugar was exponential in both the presence and the absence of insulin permits the rate constant of the efflux to be used directly as a measure of insulin activity. Park *et al.* (1961) using the efflux of L-arabinose from the heart, found that the maximal effect of insulin was a fourfold increase in the rate of efflux, which increase is similar to that found in these experiments with galactose.

The efflux of galactose from the heart in the absence of added insulin has at least three components: the galactose present in the interstitial water at the start of the wash-out; the galactose leaving the cells under the influence of the endogenous insulin-like activity of the tissue; and the transport of galactose out of the cells that takes place in the absence of all insulin-like activity. Regarding the second, Zachariah (1961) showed that there was appreciable insulin-like activity in the heart after excision, the progressive removal of which by perfusion was sufficiently slow for some activity to be present at 20–25 min, thus contributing in all experiments to the rate of efflux of galactose. This is borne out by the fact that the same seasonal change in the rate of efflux was observed in the absence of insulin as was found in the presence of the hormone—a manifestation of the seasonal change in the response of the tissue to the residual insulin-like activity.

The relation depicted in Fig. 2, between the increased rate of efflux and insulin concentration, represents the progressive activation of the carrier system involved. If this activation entails a combination, whether it be with the carrier itself, or with fixed sites in the basement membrane leading to greater accessibility of the sugar to the cell, then the insulin dose–response curve represents a saturation of the available sites of combination, and should be described by the Michaelis–Menten equation. If the increases in the fractional rate of efflux with added insulin ($R-R_0$) are regarded as velocities, and insulin as the substrate, the data may be tested for conformity with the Michaelis–Menten equation by the method of plotting due to Augustinsson (1948), where straight lines should be obtained by plotting v against v/s, i.e. ($R-R_0$) against ($R-R_0$)/I. In fact these were obtained (Fig. 4). This graphical test of the Michaelis–Menten

equation was preferred in this instance to the more usual form due to Lineweaver & Burk (1934), as the latter gave a very uneven distribution of the points along the straight line owing to the dosages of insulin used. It must be emphasized that the use of R, rather than $R-R_0$, would not be meaningful in this analysis, as it is the relation between the increase in velocity and increase in insulin concentration of the perfusate which is being tested for conformity to the Michaelis–Menten equation. R_0 represents the origin of each curve, and includes, as has already been mentioned,

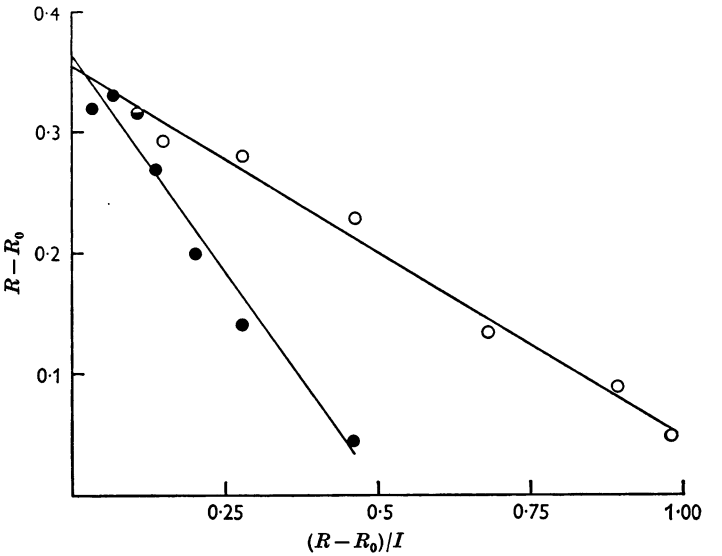

Fig. 4. The dose–response curves for the change in the fractional rate of efflux of galactose with insulin for summer (○) and winter (●) (Fig. 2), tested for conformity with the Michaelis–Menten equation by the graphical test of Augustinsson (1948). The increase in the fractional rate of efflux of galactose ($R-R_0$) is plotted against the increase in rate divided by the insulin concn. in units/litre ($(R-R_0)/I$).

the contribution due to the residual insulin activity in the heart. Figure 4 shows the typical graphical form for competitive inhibition, with the same maximum velocity for both seasons (0·353 and 0·361 ($R-R_0$) for summer and winter respectively given by the intercepts on the ordinate). The lines are those of the best fit to the points of the respective seasons. The Michaelis constants given by the slopes of the lines are, in units of insulin per litre, $0{\cdot}716 \pm 0{\cdot}006$ (7) for winter, and $0{\cdot}303 \pm 0{\cdot}020$ (7) for summer. As there is no evidence that the summer sensitivity is the maximum, these Michaelis constants should be termed apparent Michaelis constants, K_p in the nomenclature of Dixon & Webb (1958) defined by

$$K_p = K_m\left(1+\frac{i}{K_i}\right),$$

where i is the concentration of the inhibitor, and K_i the dissociation constant of the inhibitor complex. The fractional rates of efflux at three insulin concentrations after gradual reduction of the day length (Table 1) follow this same pattern, though there are obviously too few values to determine the constants.

The effect of fasting. The observation that the insulin sensitivity of the rat heart is decreased in animals fasted longer than 22 hr agrees with what has already been shown for glucose uptake in this tissue (Opie, Evans & Shipp, 1963); and also supports the presence of a serum insulin antagonist in fasting as was observed by Berman & Wertheimer (1960). The antagonism may be metabolic, for there is some evidence that a rise in the plasma, non-esterified fatty acids can depress the sensitivity of tissues to the action of insulin in promoting the utilization of glucose (Randle, 1963). Such an explanation, however, cannot account for the seasonal change in insulin sensitivity of the rat heart, as feeding on a diet rich in carbohydrate did not alter the depressed response to insulin in winter. Seasonal changes in plasma non-esterified fatty acids have been observed in rats (Barrett, 1964); the maximum levels occurred in the spring and autumn, the minimum in winter. This rules out the possibility that the consistent inhibition throughout the winter can be due to elevated levels of non-esterified fatty acids in the blood; for, as pointed out by Randle, the fatty acid inhibition is reversible with the return to normal of the fatty acid level.

Seasonal variation in insulin sensitivity. The finding that the rat heart is more sensitive to insulin in summer than in winter is in agreement with the earlier observation (Culhane, 1928) that in rabbits kept in natural lighting conditions its hypoglycaemic effect was significantly greater in summer than in winter. The records, which extended over a period of $3\frac{1}{2}$ years, showed the maximum sensitivity to be in June, and the minimum in December. A seasonal change in blood sugar was also described for rabbits with a maximum in winter and minimum in summer (Botschkareff & Grigorieff, 1929). Cori & Cori (1927) investigating the effect of fasting on ketonuria, noted that when rats were fasted for 48 hr in summer the excretion of the ketone bodies in the second 24 hr period was 6·2 mg/100 g body wt., whereas in winter it was only 1·9 mg/100 g. The change came on abruptly at the end of September and could not be reversed by keeping the rats at summer temperatures in winter. Burn & Ling (1928) simultaneously observed that the ketonuria following a diet of fat was much higher in summer than in winter. The average monthly values over the course of the year are shown in graphical form in Fig. 5, which has a close similarity to the changes in insulin sensitivity shown in Fig. 3, especially in regard to the abrupt changes in October. These authors also found that

injection of insulin augmented the small ketonuria observed in winter, and delayed the otherwise steady rise in liver glycogen found at that time. Di Maggio & Dessauer (1963) found decreased glucose tolerance, higher blood sugar and increased deposition of liver glycogen in lizards in winter, kept at constant temperature but in natural lighting conditions, and they suggest that there is a change from predominantly carbohydrate metabolism in summer to fat metabolism in winter.

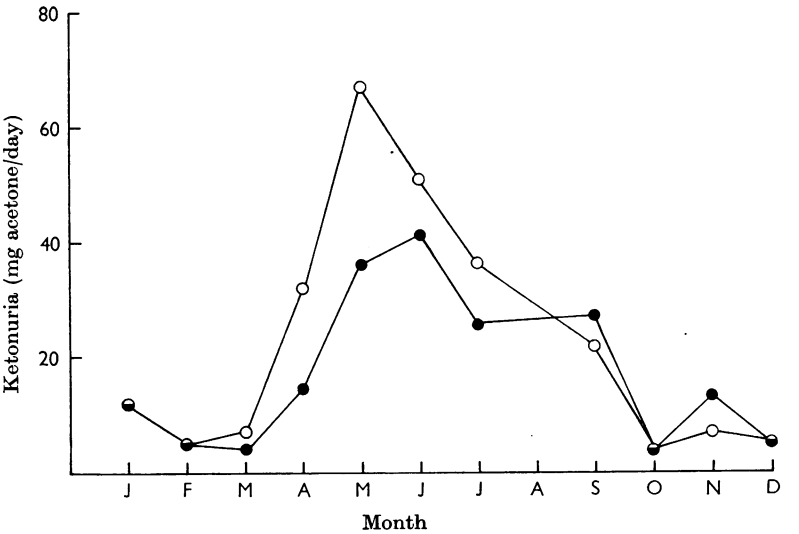

Fig. 5. The change in the ketonuria (mg acetone/day) over the course of the year in rats fed on a diet of fat. ● 2nd day of diet; ○ 3rd day of diet. The data are taken with their kind permission from Burn & Ling (1928).

The findings in the rat heart are in agreement with these observations, and taken together suggest that insulin stimulates the uptake of glucose by muscle more in summer than in winter. Furthermore, the kinetic analysis of the seasonal inhibition of insulin action already presented indicates that this differentiation could be mediated by a competitive antagonist of insulin, probably humoral in nature. The photoperiodicity of this antagonism suggests, by analogy with the photoperiodic control of sexual cycles in mammals and birds (Harris, 1960), a mediation by the hypothalamus of pituitary secretion. Spirtos & Halmi (1959) have confirmed the findings of earlier workers, describing an increased insulin sensitivity in rats following the placing of lesions in the hypothalamus. That the anterior pituitary is a source of insulin antagonism is well known from the insulin hypersensitivity which is characteristic of the hypophysectomized animal (de Bodo & Altszuler, 1958).

The role of humoral antagonists in the genesis of diabetes has received particular emphasis (Vallance-Owen, 1964), but, although their presence in normal serum has been recognized, no physiological role has been proposed for them. It is suggested on the basis of the antagonism described in this paper that the humoral antagonist of insulin action on muscle probably does represent a physiological control, for it is found to increase in activity at just that time when other observations indicate an increased deposition of fat, and a change from carbohydrate to fat metabolism. To bring about such a change the antagonism must be much less active, or probably inactive, on adipose tissue; and in fact serum and plasma albumin from both normal and diabetic subjects have been shown to antagonize the action of insulin *in vitro* on rat diaphragm but not on adipose tissue (Steinke, Taylor & Renold, 1961; Lowy, Blanchard & Phear, 1961; Alp & Recant, 1964). The antagonism described in this paper being a competitive one, its activity would be overcome only when the insulin level in the blood is high following a substantial intake of glucose. Otherwise glucose uptake by muscle will be restricted, other metabolites favoured and the resulting increased levels of glucose and insulin in the blood will increase lipogenesis. Such a mechanism would appear to be relevant in some forms of obesity, and indications or direct evidence of the combination of glucose intolerance, insulin resistance, hyperinsulinaemia and obesity are available for the three forms of hereditary obesity in mice (Christophe, 1963; Mayer, 1960), in obese children (Laplane, Etienne & Lasfargues, 1963), in obese adolescents (Vajda, Heald & Mayer, 1964) and in obese adults (John, 1929; Karam *et al.* 1963). This same combination has been shown in obese, maturity-onset diabetes (Yalow & Berson, 1960; Vallance-Owen, 1964). Thus it is tempting to see at least some forms of obesity and diabetes mellitus as the result of a prolonged exaggeration of a physiological mechanism for the control of lipogenesis, which, if it occurs early enough in life, will result in the complete exhaustion of insulin production.

SUMMARY

1. The wash-out of D-galactose from perfused rat hearts, previously loaded with the sugar, is increased by insulin. The efflux of the sugar is exponential with time in both the presence and absence of insulin, and the fractional fall per min in the heart content has been used as the measure of the rate of efflux.

2. The sensitivity of the transport process to insulin was found to be greater in summer than in winter. The relation between the increase in the rate of galactose efflux and insulin concentration conforms to the Michaelis–Menten equation for both seasons, with a competitive inhibition in the

winter. The reduced sensitivity to insulin in winter was not changed by feeding on a high carbohydrate diet.

3. A similar diminution in the insulin sensitivity of the heart can be produced by the gradual shortening of the day length of artificial light from 12 to 8½ hr over a period of 6 weeks; but not by an abrupt change from 12 to 8½ hr day.

4. Hearts from rats fasted for longer than 22 hr were also found to have a reduced sensitivity to insulin.

5. The role of the hypothalamic–pituitary axis in the production of a competitive antagonist of insulin action on muscle is discussed, together with the implication that such an antagonism could result in increased lipogenesis by the diversion of glucose from muscle to adipose tissue.

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