Summary. Rabbits injected with streptococcal extracellular protein (SEP) developed degenerative and infiltrative lesions in the heart and liver, with coagulation necrosis and multinucleated giant cells in the latter organ. The majority of these rabbits also showed elevated levels of glutamic-oxalacetic transaminase, or of sorbitol dehydrogenase, and all showed elevated serum lipids. These biochemical indications of cell injury could be found within 2 to 4 hours after a first injection of SEP. No such biochemical or pathologic effects were found following an injection of heated SEP or a commercially available streptokinase–streptodornase preparation from group C streptococcal culture. In a preliminary fractionation of SEP all the activity was found in a fraction not adsorbed to DEAE cellulose at pH 7.4 (0.05 *M* PO_4). The active fraction contained at least five antigens and four of the known streptococcal enzymes. Injection of extracts of sonically disrupted streptococci produced similar biochemical and pathologic changes. There was some cross-reacting material between the sonicates and anti-SEP serum.

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Received, Dec. 4, 1967. P.S.E.B.M., 1968, Vol. 127.

Hyperphagia in the Insulin-Treated Rat* (32909)

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We have described recently certain metabolic effects of hyperphagia in the hypothalamic-hyperphagic rat (1). In the early dynamic phase of hyperphagia, increased body weight gain appeared to be a direct effect of increased energy intake not balanced by the small increase of spontaneous activity; increased lipogenesis and decreased lipolysis *in vitro* were observed with no evidence of altered thyroid activity. In the later static phase of hyperphagia, spontaneous activity was not increased, metabolic patterns were altered in the direction of increased lipogenesis and decreased lipolysis, and there appeared to be thyroid hypofunction. In 1940, MacKay *et al.* (2) demonstrated that protamine zinc insulin (PZI) increases food intake and body weight gain. This observation has been amply confirmed by several groups of investigators and the potent property of PZI is apparent from the observation of Beaton *et al.* (3) that PZI overcomes the

* Supported in part by a grant from the Medical Research Council of Canada. Taken from a thesis submitted by K. K. May to the Faculty of Graduate Studies, University of Western Ontario, in partial fulfillment of the requirements for the degree of Master of Science.

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marked hypophagia of rats fed a low-protein diet as well as inducing hyperphagia in animals fed a control diet. The present experiments were undertaken to compare in part, the metabolic mechanisms of increased fat deposition in PZI-treated rats with the previously reported mechanisms induced by hypothalamic lesions.

Materials and Methods. Adult male rats of the Wistar strain, with an initial average body weight of about 200 gm, were housed in individual, wire-screen cages at an environmental temperature of 24±1°C with 12 hours' light and 12 hours' darkness each day. The animals were provided *ad libitum* with a synthetic high-carbohydrate diet (20% casein, 64% sucrose, 10% corn oil, 4% salts mixture, 2% alphacel by weight; caloric value 4.1/gm). The diet contained vitamins, choline, and inositol in quantities adequate for optimal growth.

Two experiments were carried out in each of which 7 rats were injected subcutaneously daily with 0.9% aqueous sodium chloride and 7 rats with protamine zinc insulin (PZI) at a dosage of 2 units/100 gm of body weight. Food intake and body weight were measured at regular intervals. Lipogenesis *in vitro* was determined by incubating acetate-1-^{14}C with adipose tissue (epididymal fat pad) according to Baruch and Chaikoff (4). Incorporation of glucose-U-^{14}C into lipid and its oxidation to $^{14}CO_2$ in adipose tissue *in vitro* were determined by the procedure of Chernick *et al.* (5). Lipolysis was estimated *in vitro* by free fatty acid release of adipose tissue as described by Beaton *et al.* (6). The concentration of total crude fatty acids in adipose tissue (epididymal fat pad) was determined by a modified Liebermann procedure (7).

In the first experiment, animals were treated for a period of 16 days with food intake measured twice daily at 12-hour intervals. At the end of this time and 24 hours after the last PZI injection, both epididymal fat pads were removed under anesthesia (pentobarbital sodium, intraperitoneal, 3 mg/100 gm of body wt.). One pad was used for the measurement of acetate-1-^{14}C incorporation into lipid; the remaining pad was used to determine rate of lipolysis. In the second experiment, animals were treated for a period of 14 days. An epididymal fat pad from each rat was used to determine lipogenesis from glucose-U-^{14}C and the oxidation of this substrate to $^{14}CO_2$. The concentration of total crude fatty acids was determined in the remaining pad of each animal.

Results. Observations of daily food intakes and body weights in the first experiment are recorded in Fig. 1. A gradual increase of food

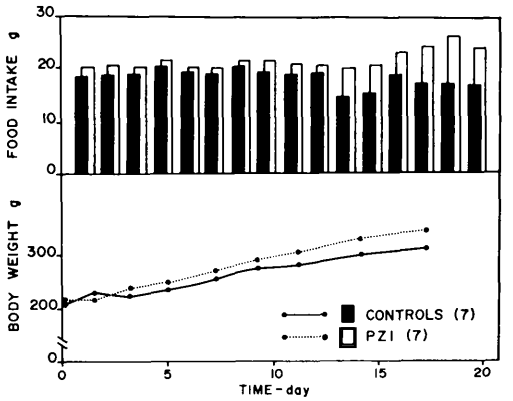

FIG. 1. Average daily food intakes and body weights of saline-injected and protamine-zinc-insulin injected (2 units/100 gm of body wt.) rats. Data are averages of 7 rats/group.

intake with each successive day of PZI treatment was noted; the effect of PZI was most evident from day 11 onward. Average daily food intakes over the 16-day period were 21.0 ± 0.30 and 18.0 ± 0.90 gm/rat/day for the PZI and control groups, respectively ($p < 0.01$). The body weight gain of PZI-treated rats exceeded that of control animals ($p < 0.01$).

As shown in Fig. 2, PZI-treated rats consumed 34% of their food intake during daylight hours whereas controls consumed 24% during the same period. As shown in Table I, lipogenesis in adipose tissue *in vitro* was significantly increased in PZI-treated rats whereas lipolysis was not significantly altered.

The results of the second experiment are shown in Table II. Lipogenesis from glucose-U-^{14}C *in vitro* was significantly increased in PZI-treated rats but the apparent oxidation of glucose-U-^{14}C to $^{14}CO_2$ was not signifi-

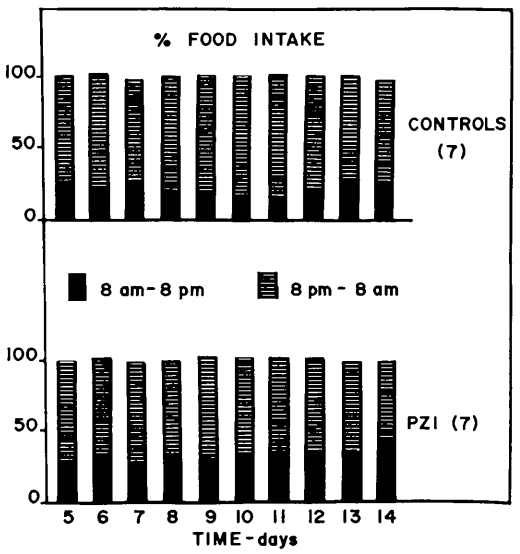

FIG. 2. Feeding patterns of saline-injected and protamine-zinc-insulin injected (2 units/100 g body weight) rats. Data are averages of 7 rats/group.

TABLE I. Acetate-1-^{14}C Incorporation into Lipids and Free Fatty Acid Release *in Vitro* in Adipose Tissue of PZI-Injected Rats. (results expressed as mean ± SEM for 7 rats)

Group	^{14}C-lipids (cpm /gm of tissue)	Free fatty acid release (μmol/100 mg of tissue/3 hours)
Control	1700 ± 503	1.2 ± 0.31
PZI	10979 ± 3387	1.0 ± 0.17
Probability, p	<0.02	

cantly altered. There was no difference in the concentrations of total crude fatty acids in adipose tissue between the two groups.

Discussion. It is apparent that in certain measurements, the metabolic effects of daily PZI injection resemble those of hypothalamic hyperphagia both in the dynamic and static phases (1). In both forms of hyperphagia, there are: increased lipogenesis by adipose tissue *in vitro* but without alteration in oxidation of glucose-U-^{14}C to $^{14}CO_2$ *in vitro*. Our observation of increased lipogenesis is in agreement with the reports of others (8–10). In hypothalamic hyperphagia there is decreased lipolysis *in vitro* in adipose tissue (1); this was not observed in the case of PZI-hyperphagia nor was there any alteration in fat content of the adipose tissue. In both cases, the hyperphagia is associated with a trend toward equal day: night eating and excessive body weight gain; this increased weight gain has been shown to be due in large part to excessive deposition of body fat in hypothalamic hyperphagia (11) and in PZI hyperphagia (3). The dynamic phase of hypothalamic hyperphagia has been shown to be associated with an increased spontaneous running activity (1); however, a decrease of spontaneous running activity has been reported in PZI-treated rats (3). Whereas there is evidence of thyroid hypofunction in the static (but not dynamic) phase of hyperphagia (1), resting oxygen consumption is normal in rats injected daily with PZI (3).

Although the mechanism of action of PZI on food intake may be related to its effect on blood levels of glucose via a glucostatic regulation (12), it has been reported recently that PZI may act also via thermostatic regulation (13). Whatever the mechanism, it is apparent that PZI is an effective hyperphagia-inducing agent leading to excessive body weight gain and deposition of body fat. Although some metabolic patterns associated with PZI treatment resemble those of hypothalamic hyperphagia (increased lipogenesis by adipose tissue *in vitro*, increased body fat deposition) others appear to differ

TABLE II. Glucose-U-^{14}C Incorporation into Lipids and its Oxidation to $^{14}CO_2$ *in Vitro* in Adipose Tissue and the Fat Content of Adipose Tissue in PZI-Injected Rats. (results expressed as mean ± SEM for 7 rats)

Group	^{14}C-lipids (cpm/gm of tissue)	Recovered as $^{14}CO_2$ (%)	Total crude fatty acids (%)
Control	1158 ± 54.23	1.2 ± 0.19	21 ± 1.73
PZI	2659 ± 219.41	1.5 ± 0.20	27 ± 3.32
Probability, p	<0.001		

(spontaneous activity, resting metabolism, lipolysis by adipose tissue *in vitro*).

In the interpretation of these results, some caution must be exercised. Alterations in energy expenditure (spontaneous activity, resting metabolic rate) may be secondary features not having a direct cause: effect relationship in the observed increased deposition of body fat. Further, the observed increased lipogenesis *in vitro* may represent adaptation to an excessive calorie intake rather than being a causative factor. However, it would seem reasonable to conclude that the metabolic mechanisms of increased fat deposition differ in some characteristics between these two forms of hyperphagia.

Summary. Protamine zinc insulin (PZI) was administered daily to male rats by subcutaneous injection (2 units/100 gm of body weight) and comparisons were made with saline-injected control animals. As a consequence of PZI administration, the following observations were made: hyperphagia, increased body weight gain, increased lipogenesis from acetate-1-^{14}C and glucose-U-^{14}C, and no alteration in oxidation of glucose-U-^{14}C to $^{14}CO_2$, in lipolysis *in vitro*, nor in the fat concentration of adipose tissue. These results are compared with those reported previously with hypothalamic-hyperphagic rats and it is concluded that the metabolic mechanisms of increased fat deposition differ between the two forms of hyperphagia.

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Received Dec. 4, 1967. P.S.E.B.M., 1968, Vol. 127.

Deficient Immunologic Functions of NZB Mice* (32910)

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The role of the thymus in the spontaneous autoimmune hemolytic anemia of the NZB mice is still unknown. Thymic lesions have been described in these mice (1,2) although in some cases there is no direct correlation between the appearance of signs of disease and thymic lesions. This was especially noted in animals in which the disease was transferred with spleen cells (3). The effects of thymectomy are even more contradictory since both delay (3) and more precocious development of the disease has been reported in neonatally thymectomized mice (4). The NZB thymus grafts can transfer the disease to thymectomized CBA/T6 mice that do not develop autoimmune phenomena spontaneously although thymus cells from sick NZB mice are unable to transfer the disease to normal young animals (3,4). Although in-

* This work was supported by grants from U. S. Public Health Service (5T1 GM 131302, HE-02085, E-0798) and the National Foundation.

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