detect such decreases in tissues obtained from young and old humans. Ehrlich *et al.* (*13*) also found no significant changes in the 5-methylcytosine content of human fibroblasts allowed to undergo approximately 10 to 15 doublings. However, from our data, this may not have represented a sufficiently long time span for measurable differences to be apparent in human cells.

The decreases in 5-methylcytosine that we have observed in cultured cells from three different species may account for aberrant gene expression in aging cultures (*14*). However, it remains to be seen whether this represents a response to the culture environment or has any significance in vivo.

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20 December 1982; revised 9 February 1983

## Glucose Stimulation of the Antilipolytic Effect of Insulin in Humans

Abstract. *Dose-response studies of the inhibition of lipolysis by insulin in isolated human adipocytes were conducted with the use of a sensitive bioluminescent assay of glycerol release. The addition of glucose to the incubation medium was associated with an increase in insulin sensitivity and an increase in the maximum insulin effect. The results suggest that glucose plays an important role in regulating the antilipolytic action of insulin in humans.*

Insulin inhibits lipolysis and stimulates the cellular uptake and metabolism of glucose in adipose tissue. The antilipolytic effect of insulin in the rat is clearly independent of any action of the hormone on glucose metabolism (*1*). Dose-response studies of the inhibition of lipolysis by insulin in isolated rat fat cells indicate that although glucose does not influence the action of insulin on basal lipolysis (*2*), it may completely abolish the insulin inhibition of catecholamine-stimulated lipolysis (*3*). It is unclear whether glucose alters the antilipolytic effect of insulin in humans. In human fat cells insulin often fails to inhibit glycerol release (*4*). This may be due to the technical difficulties of estimating glycerol release, which is generally used as an index of the rate of lipolysis (*5*) and is about ten times more rapid from rat than from human adipocytes (*5, 6*). We recently developed a bioluminescent technique for the determination of glycerol release from fat cells. This technique is 100 times more sensitive than the commonly used enzymatic, fluorometric methods (*7*). In the present study we used the bioluminescent technique to determine whether there is an interaction between glucose and the antilipolytic effect of insulin in man. We prepared isolated human fat cells and measured the rate of glycerol release in vitro (*8*).

Figure 1 shows the dose-response curve for the effect of insulin on basal and isoprenaline-induced lipolysis. When either a submaximal effective or a maximal effective concentration of isoprenaline was used, we observed a dose-dependent influence of glucose on the antilipolytic effect of insulin. Furthermore, the two different isoprenaline concentrations produced almost identical results. When fat cells were incubated in a glucose-free medium, they were unresponsive to insulin added at physiological concentrations. A slight inhibition of lipolysis was observed in the presence of 2500 μU of insulin per milliliter. The addition of glucose (1 mg/ml) to the medium induced a shift to the left in the dose-response curve for insulin, but a plateau was never reached. A higher concentration of glucose (2 mg/ml) was associated with a further shift to the left. A maximum antilipolytic effect of insulin was obtained with the higher insulin concentrations tested. The concentration of insulin that produced a half-maximum effect ($ED_{50}$) in the presence of 2 mg of glucose per milliliter was about 5 μU/ml in both experiments with isoprenaline.

The addition of glucose to the incubation medium did not induce a shift to the left of the dose-response curve in the basal state (Fig. 1). The basal rate of lipolysis was inhibited by insulin at physiological concentrations, with and without the presence of glucose in the incubation medium. The $ED_{50}$ for insulin was 2.5 μU/ml with 1 or 2 mg of glucose per milliliter. However, the amplitude of the dose-response curve was greater with than without glucose. This indicates that glucose may stimulate the maximum antilipolytic effect of insulin (insulin responsiveness) in the basal state. Further studies (*9*) showed that the addition of glucose (2 mg/ml) to the incubation medium was followed by an increase in insulin responsiveness from 40 to 56 percent ($P < .01$). Neither the isoprenaline-induced rate of glycerol release nor the basal rate were influenced by glucose (Fig. 1).

The interactions among glucose, insulin and lipolysis in human fat cells observed in the present study were markedly different from those described in rat adipocytes (*1–3*). In the rat the inhibitory effect of insulin may be either uninfluenced or abolished by the presence of glucose in the incubation medium. Our data indicate that, in man, glucose stimulates the antilipolytic effect of insulin. Furthermore, the present data suggest that glucose may regulate the antilipolytic effect of insulin in human adipocytes in two ways. When lipolysis is maximally or submaximally stimulated by catecholamines, insulin sensitivity is increased by glucose. In the basal state glucose does not influence insulin sensi-

tivity but enhances insulin responsiveness.

Although we cannot determine from the present experiments the mechanisms by which glucose stimulates the antilipolytic effect of insulin, it can be inferred from previous studies (*10*) that alterations in insulin receptor binding produce changes in insulin sensitivity, and that alterations at the postreceptor level produce changes in insulin responsiveness. It is therefore possible that glucose influences the antilipolytic effect of insulin by separate mechanisms in human fat cells. One effect of glucose may be closely related to the insulin receptor. This may be important for stimulated lipolysis. Another effect may be localized at the intracellular steps in insulin action. This is important for basal lipolysis. Alternatively, insulin may inhibit basal and catecholamine-stimulated lipolysis by separate processes, both of which may then be stimulated by glucose. Interactions between glucose and insulin may not be unique to antilipolysis. In a study of glucose transport and lipogenesis in isolated human adipocytes, Pedersen and Hjöllund (*11*) found that compared with experiments in which they used trace amounts of glucose in the medium, higher glucose concentrations (0.5 to 2 m*M*) resulted in an increased insulin sensitivity but a decreased maximum insulin effect.

The ability of insulin to inhibit lipolysis in fat cells has been recognized for two decades (*12*). The present results indicate that glucose may play an important role in regulating the antilipolytic action of insulin in human fat cells in vitro. Such an effect may also occur in vivo. We have recently observed that there is a marked increase in the sensitivity of the antilipolytic effect of insulin in human fat cells obtained 1 hour after glucose ingestion (*13*).

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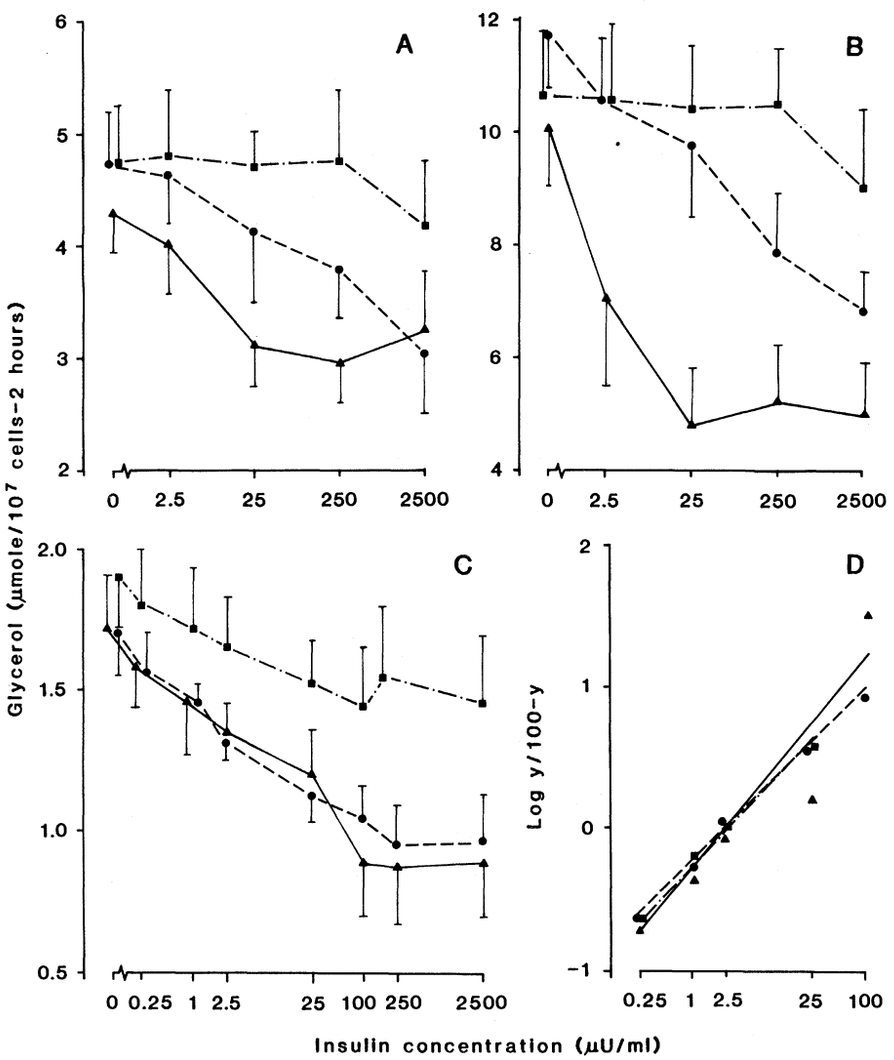


Fig. 1. Insulin inhibition of (A and B) isoprenaline-induced lipolysis and (C and D) basal lipolysis. Fat cells were incubated in duplicate with (■) 0, (●) 1 mg, or (▲) 2 mg of glucose per milliliter and insulin was added at various concentrations. (A) Experiments with medium containing a submaximum effective concentration ($6 \times 10^{-7}$ *M*) of isoprenaline ($N = 4$). (B) The medium contained a maximum effective concentration ($6 \times 10^{-6}$ *M*) of isoprenaline ($N = 6$). (C) The medium was free of isoprenaline (basal lipolysis) ($N = 10$). (D) Insulin sensitivity in the basal state ($N = 10$). The relation between insulin concentration and insulin effect was plotted linearly. The ascending part of the dose-response curve was transformed to a log-logit plot where $y$ denotes insulin effect at a particular insulin concentration as a percentage of the maximum insulin effect (*14*). The position of the line in the diagram was determined by linear regression analysis according to the method of least squares. The concentration of insulin producing a half-maximum effect is obtained where the line crosses the abscissa at 0. The results represent means ± standard error. The rate of glycerol release remained linear for at least 4 hours under all experimental conditions.

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9. Fat cells were prepared and incubated in dupli-

cate as in (*8*). The medium contained 0 or 2 mg of glucose per milliliter and various insulin concentrations (0 to 2500 μU/ml), but no isoprenaline. Insulin responsiveness was calculated as the percentage inhibition of basal glycerol release at the maximum effective concentration of the hormone. The results of 15 experiments (mean ± standard error) showed that insulin responsiveness was 40.3 ± 3.2 percent without and 56.1 ± 5.7 percent with glucose present ($P < .01$, paired $t$-test).


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15. This study was supported by grants from the Swedish Medical Research Council, the Karolinska Institute, the Swedish Diabetes Association, the Swedish Medical Association, and the Osterman, Nilsson, Hierta, Folksam, and Groschinsky foundations.

25 January 1983


## Neural Crest Cells Contribute to Normal Aorticopulmonary Septation


Abstract. *By analyzing the hearts of quail-chick chimeras, it was found that neural crest cells at the level of occipital somites 1 to 3 migrate to the region of the aorticopulmonary septum. Bilateral removal of this neural crest population prior to migration causes malformation of the aorticopulmonary septum resulting in common arterial outflow channels or transposition of the great vessels.*


Transposition of the great vessels and other defects in the formation of the aorticopulmonary septum represent profound disturbances of the basic architecture of the heart. Aorticopulmonary septal defects are clinically referred to as conotruncal abnormalities and include transposition of the great vessels, overriding aorta, double-outlet right ventricle, and persistent truncus arteriosus (*1*). Transposition of the great vessels is the most common of these defects in humans and is found in 12 percent of neonates with congenital heart defects (*1*). The pathogenesis of transposition of the great vessels and related aorticopulmonary septal defects is unknown (*1*). The present study shows that depleting the heart of cells derived from occipital neural crest cells can result in aorticopulmonary septal defects.

We recently reported the removal of the parasympathetic postganglionic innervation to the heart (*2*). Cardiac parasympathetic postganglionic neurons arise from the occipital region of neural crest and migrate toward the heart while it is a simple tube. In that study, the neural crest population that seeds the cardiac ganglia was removed prior to its migration. In the present study, we demonstrate that defects in the aorticopulmonary septum result when the neural crest region containing presumptive cardiac ganglion cells is removed.

Fertile White Leghorn or Arbor Acre chicken eggs and Japanese quail eggs were incubated for 24 to 30 hours in a humidified atmosphere at 37.5°C. Both quail (donor) and chick (host) eggs were opened and prepared for microsurgery (*3*) at stage 9 of development (*4*). The procedure described by Narayanan and Narayanan (*5*) was followed for the interspecific transplantation of cranial neural crest between quail and chick embryos. The chick neural fold over somites 1 to 3 was excised bilaterally with a modified Wenger vibrating needle (*5*). The neural fold consists of the presumptive dorsal part of the neural tube, the neural crest, and some adjacent surface ectoderm (*6*). Two different experiments were performed using the chick embryos with bilaterally excised neural folds. In the first series, chick neural fold was replaced with homotypic quail neural fold. In the second series, the chick neural fold was removed with no futher manipulation of the embryo. After surgery, the eggs were sealed, returned to the incubator, and allowed to develop for an additional 4 to 14 days (total development of 5 to 15 days). Embryos that received sham operations were processed in parallel with each group of microsurgically manipulated embryos. Embryos were fixed by perfusion through the left ventricle with 10 percent neutral buffered Formalin. Appropriate portions of the embryos were embedded in paraffin and processed for serial light microscopy. Feulgen Rossenbeck staining was used for chimeras and hematoxylin and eosin staining for control embryos or embryos with extirpations. All of the embryos described in the results were grossly normal at the time of perfusion.

Thirteen quail-chick chimeras ranging in total incubation age from 6 to 9 days were examined. Clusters of quail cells could be found near the branchial arch arteries and truncus arteriosus of 6-day chick embryos. The developing truncal septum was composed of chick mesenchymal cells interspersed with quail mesenchymal cells (Fig. 1). By day 7 the truncal septation was complete in the chick embryo. Quail and chick mesenchymal cells could be recognized from days 7 to 9 in the aorticopulmonary septum and in the tunica media of the aorta and pulmonary trunk. Other clusters of quail cells, which were identified as developing cardiac ganglia, were segregated from the cells associated with the developing tunica media as reported previously (*2*).

Sixteen embryos with extirpation of

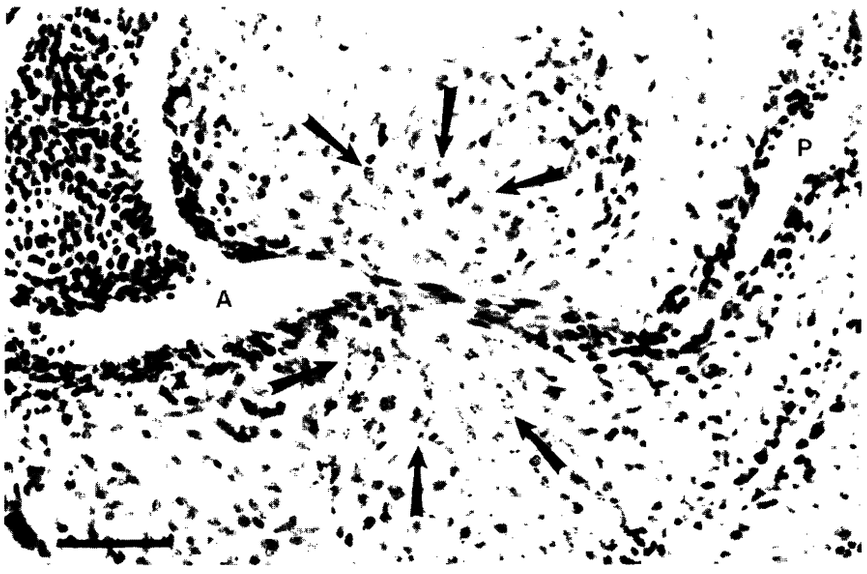


Fig. 1. Cross section of the truncal region during septation in a 6-day chick embryo with a bilateral quail neural fold transplant at the level of somites 1 and 2 at stage 10. Typical quail cells (arrows) can be seen in the region of the developing septum. *A*, aorta; *P*, pulmonary trunk. Scale bar, 50 μm.