the "fatty liver was consistently present" in human cases of hypercorticism he reviewed (3); our own experience includes many cases with fatty liver, and many without. Perhaps some other factor added to high cortisone levels, neither of which would produce the fatty liver by itself, is present.

Summary. Cortisone acetate, 6.25 mg/day, consistently produces elevation in rat serum glycerides and cholesterol, and in hepatic lipid glycerides other than phospholipid. Hepatic phospholipid and cholesterol levels are not affected. The question whether this represents a primary effect on the liver or a secondary effect due to mobilization of peripheral fat is discussed.

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Insulin Secretion from the Isolated Pancreas in Absence of Insulinogenesis: Effect of Glucose.* (28791)

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Although glucose stimulates the release of insulin from the islets of the pancreas, it is not clear whether it affects insulinogenesis primarily or acts directly on the secretion mechanism. Humbel *et al*(1) found that glucose-C^{14} can serve as a precursor for synthesis of insulin-C^{14} in fish islets, suggesting that high concentrations of glucose could stimulate insulinogenesis by providing substrate. In addition, Seltzer(2) has explained differences in the patterns of insulin secretion after administration of glucose and tolbutamide in the dog as a result of the stimulation of insulinogenesis by glucose.

On the other hand, an increased secretory process can be visualized(3,4) and depletion of islet cell granules is readily demonstrated after administration of glucose. Although these studies indicate that insulin secretion is accelerated by glucose, they still do not establish whether stimulation of insulin synthesis by glucose was the initial event.

We have previously demonstrated that glucose stimulates the release of immunochemically measurable insulin from the isolated perfused pancreas of the rat and that atten-

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dant islet cell degranulation occurs(5). Since the experimental preparation is extracorporeal and the assay is unaffected by metabolic inhibitors, it is possible to study insulin secretion under circumstances in which all protein synthesis is inhibited with 2,4-dinitrophenol (DNP).

Methods. Pancreas and adjacent spleen, stomach and duodenum were surgically removed from adult male rats weighing 250 g which had been fasted overnight and anesthetized with sodium pentobarbital. The preparation was immediately transferred to the perfusion apparatus, modified after Anderson and Long(6) as previously described (5), and continuously perfused with 160 ml of a mixture of fresh rat blood and 2% gelatin (1:1 v/v) for 3 hours. When employed, DNP was added to the perfusates at zero time. Valine-C^{14}, 1,600 counts/min/ml perfusate, was added after the first hour in all experiments. Glucose, 100 mg/100 ml, was also added after the first hour in some experiments but omitted in others. At 3 hours, the tail and body of the pancreas, weighing approximately 1 g, were removed and homogenized in 5% cold trichloracetic acid (TCA), 25 vol/g tissue, containing carrier valine. After centrifugation, the protein precipitate was washed sequentially in cold TCA, boiling TCA, alcohol, alcohol-ether, and ether, and was counted on planchets by a thin-end window Geiger-Muller counter.

Insulin was measured by an insulin-I^{131} immunochemical assay previously described (5,7); porcine insulin-I^{131} was incubated in a mixture of urea, albumin, and glycine buffer with sufficient antibody (originally prepared in guinea pigs against porcine insulin) to bind 80–85% of the isotope. After addition of carrier globulin, the bound insulin was removed by preferential precipitation with Na_2SO_4. Free insulin-I^{131} in the supernatant increases with an increase of unlabeled insulin present in added perfusate. Since rat insulin was not available as a standard, results were reported empirically as the per cent increase in free insulin-I^{131} with time, taking the value of the perfusate at zero time as the zero reference point. Neither glucose nor DNP interfered with the assay when added to the perfusate in the concentration ranges employed.

TABLE I. Incorporation of Valine-C^{14} into Total Pancreatic Protein.

Exp No.	Glucose (mg/100 ml)	DNP	Valine-C^{14} incorporated (cpm/pancreas)
1	0	0	16,000
2	0	0	18,000
3	100	0	14,000
4	100	0	12,800
5	100	0	13,300
6	100	0	9,500
7	0	1×10^{-4}	22,000
8	100	1×10^{-4}	3,300
9	100	1×10^{-4}	15,400
10	100	1×10^{-4}	3,240
11	0	5×10^{-4}	3
12	100	5×10^{-4}	30
13	100	5×10^{-4}	0
14	100	5×10^{-4}	49

Results and discussion. Previous studies showed that in the absence of added glucose, endogenous glucose from the perfusate is rapidly utilized by the preparation and falls to less than 15 mg/100 ml after 2 hours; these concentrations are insufficient to stimulate insulin secretion. Detectable insulin does not appear in the circulating perfusate until the glucose levels are maintained above 45–50 mg/100 ml(5).

Table I shows that after 3 hours protein synthesis in the pancreas, regardless of glucose concentration, was only partially inhibited with 1×10^{-4} M DNP but could be completely inhibited at concentrations of 5×10^{-4} M. It was noted that the motility of the tissue after 3 hours was consistently reduced by the latter concentration of DNP. Therefore, to establish the viability of the tissue, oxygen consumption was evaluated by measuring the arteriovenous difference in O_2, using the oxygen electrode. In all experiments, O_2 consumption continued in the presence of 5×10^{-4} M DNP, throughout the 3-hour perfusion period.

The effect of glucose, added after one hour to raise the circulating insulin levels in the presence or absence of 5×10^{-4} M DNP, was measured in 20 perfusion experiments (Fig. 1) including the 4 containing valine-C^{14} previously shown in Table I. By the third hour, glucose caused marked stimula-

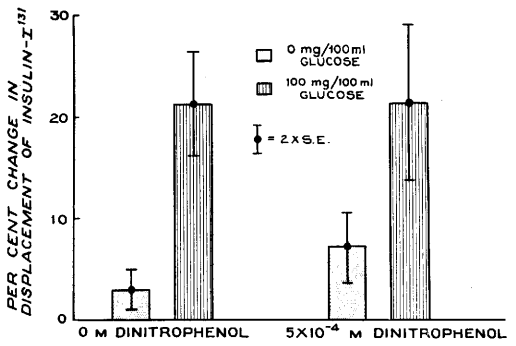

FIG. 1. Effect of 5×10^{-4} M dinitrophenol on glucose stimulation of insulin secretion from isolated perfused pancreas of the rat. Insulin levels were measured immunochemically at 3 hr. Glucose when employed was added at 1 hr. N = 5 for each group.

tion of insulin release from the isolated pancreas. In the presence of 5×10^{-4} M DNP, glucose stimulation of insulin release was unimpaired, despite the fact that all protein synthesis in the tissues was blocked. DNP alone caused the appearance of small amounts of insulin in the circulating perfusates, possibly as a result of cell necrosis caused by nonspecific effects of this agent on tissue metabolism but, as shown in Fig. 1, this did not account for the large amounts of insulin observed when glucose and DNP were both present.

The demonstration that stimulation by glucose of insulin secretion from the pancreas is unimpaired under conditions in which protein synthesis is totally inhibited indicates that glucose can act directly on the secretory process. Although glucose may also directly stimulate insulinogenesis *de novo* from amino acids, apparently this is not an essential requirement for stimulation of secretion. The results of these experiments do not exclude an influence of glucose on the synthesis of insulin from a polypeptide precursor.

The mechanism by which glucose affects insulin stimulation is unknown. Since other metabolizable sugars such as mannose, and to a lesser extent fructose, stimulate insulin secretion and the poorly metabolizable sugars galactose, 2-deoxyglucose and xylose do not (5), a common metabolite or product of metabolism of carbohydrates may be the important factor. That adenosine triphosphate is this factor is unlikely since its level would presumably be reduced by the uncoupling action of DNP when employed in concentrations adequate to inhibit protein synthesis.

Summary. The effect of dinitrophenol (DNP) on glucose stimulation of the release of immunologically measurable insulin was studied in the isolated perfused pancreas of the rat. In a 3-hour perfusion at 5×10^{-4} M DNP, protein synthesis, as measured by incorporation of valine-C^{14} into total pancreatic protein, was completely inhibited. The release of insulin by glucose, however, was unimpaired. Results indicate that glucose (or a product resulting from glucose metabolism) can stimulate insulin secretion directly and that a prior stimulation of insulinogenesis from amino acids is not required.

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