STUDIES ON THE DIFFERENTIAL RESPONSE TO INSULIN ON THE STIMULATION OF AMINO ACID INCORPORATION INTO PROTEIN IN ISOLATED HEPATOCYTES CONTAINING DIFFERENT LEVELS OF GLYCOGEN*

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SUMMARY

Effect of insulin on amino acid incorporation into protein by isolated rat liver hepatocytes was studied. A two to three-fold increase in the incorporation of U-^{14}C-Leucine and U-^{14}C-Phenylalanine into protein by insulin (100 μUnits) was observed in isolated hepatocytes containing high glycogen. This effect was abolished by the addition of glucagon (3 x 10^{-6}M). No stimulation in amino acid incorporation by insulin was observed when isolated hepatocytes contained low or no glycogen. Electron micrographs of incubated cells show that in the presence of insulin more normal parallel strands of polyribosomes are maintained as compared to control cell preparation.

Insulin is generally accepted as a protein-anabolic hormone. The rate of protein synthesis measured in several ways is subnormal in tissues of diabetic animals and can be restored to normal by treatment of animals with insulin (1, 2). However, the primary site of this metabolic defect is not known. In the present studies we report an interrelationship between glycogen levels and stimulation of amino acid incorporation into protein by insulin in isolated hepatocytes.

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MATERIAL AND METHODS

Male rats weighing 180-200 g, fed or fasted (6-8 hrs; 16-18 hrs), were used in all studies reported here. All rats were maintained on Purina Laboratory Chow and tap water fed ad libitum. The rats received their food in a dish that was placed on the floor. It was previously observed (3) that liver glycogen was significantly increased in animals that had easy access to the food as compared to those rats that had to obtain their food from suspended wire baskets. Rat liver cells were isolated by collagenase *in vitro* perfusion technique as described previously (4). Approximately 55-70 mg cells were incubated in 3 ml of Umbreit Ringer, 25 mM $NaHCO_3$ buffer (5) containing 7.5 mM glucose, 5 mM lactate and 5 mM amino acids (containing 0.5 μC of U-^{14}C-leucine or U-^{14}C-phenylalanine) and with various concentrations of hormones at 37°C and at 90 oscillations per minute for 1-3 hrs. The vials were gassed with 95% O_2 and 5% CO_2 for 5 min. at time zero and after each 1 hr of incubation. Insulin, 50 μUnits, was added on time zero and 25 μUnits was added at the end of one and two hours. Glucagon (10^{-6}M) was added at time zero, one and two hrs. At the end of the incubation period the reaction was stopped by the addition of trichloracetic acid and radioactivity in the protein was assayed as described previously (6,7). Cellular glycogen was precipitated by the method of Good *et al.* (8), hydrolyzed and assayed by glucose oxidase method (9). The ultrastructure studies were carried out by thin section electron microscopy on the Sieman IA polarizing electron microscope. Cells after incubation (1 hr) were fixed overnight at 0°C with 2% glutaraldehyde in 0.1 M Na phosphate (pH 7.3) buffer. The cells were rinsed twice with 0.1 M Na phosphate buffer and post-fixed with 2% osmium tetroxide in 0.05 m Na phosphate buffer for one hour at 0°C. They were rinsed again in 0.05 M Na phosphate buffer and dehydrated in a graded ethanol series to 100% ethanol. Infiltration was completed with propylene oxide and samples were embedded in Epon-812 resin. Thin sections were cut with LKB ultratome. They were stained with 2% uranyl acetate in absolute ethanol and post-stained with Reynolds lead citrate (10).

RESULTS AND DISCUSSION

The incorporation of U-^{14}C-leucine and U-^{14}C-phenylalanine into protein by isolated hepatocytes is summarized in Table I. The incorporation of labeled leucine and phenylalanine increased proportionately with incubation time in the presence of 7.5 mM glucose, 5 mM lactate and 5 mM amino acids mixture. Treatment with insulin (100 μUnits) showed a progressive increase in the incorporation of these amino acids into protein at various incubation periods studied, reaching an increase of almost three-fold by the end of three hours. Addition of glucagon (3×10^{-6}M) had no effect on the incorporation of these amino acids into protein. Insulin-stimulated amino acid incorporation into protein by isolated hepatocytes was abolished by glucagon when cells were incubated with combinations of insulin (100 μUnits) and glucagon (3×10^{-6}M). No stimulatory effect by added insulin was observed in isolated

TABLE I

EFFECT OF INSULIN AND GLUCAGON ON THE INCORPORATION OF U-^{14}C-LEUCINE AND U-^{14}-PHENYLALANINE INTO PROTEIN IN ISOLATED HEPATOCYTES FROM FED RATS* (dpm/mg protein)

	U-^{14}C-LEUCINE	U-^{14}C PHENYLALANINE	U-^{14}C-LEUCINE	U-^{14}C-PHENYLALANINE	U-^{14}C-LEUCINE	U-^{14}C-PHENYLALANINE
PERIOD OF INCUBATION	1 HR		2 HR		3 HR	
CONTROL	1120 ± 150	810 ± 92	1880 ± 200	1380 ± 110	3260 ± 340	1760 ± 190
CONTROL + INSULIN (100 μUnits)	2080 ± 190	1560 ± 160	4260 ± 410	2975 ± 330	8800 ± 1680	4650 ± 360
CONTROL + GLUCAGON (3×10^{-6}M)	1280 ± 180	925 ± 110	1960 ± 210	1520 ± 130	3480 ± 400	2100 ± 240
CONTROL + INSULIN (100 μUnits) + GLUCAGON (3×10^{-6}M)	1320 ± 175	1056 ± 170	2130 ± 240	1680 ± 160	3650 ± 420	2260 ± 280

Hepatocytes were prepared as described previously (4). Isolated hepatocytes had initial glycogen levels in the range of 185 ± 35 μmoles glucose/g. Approximately 55-75 mg of cells were incubated for 1-3 hours in 3 ml of Umbreit Ringer bicarbonate buffer (5) containing 7.5 mM glucose, 5 mM lactate and 5 mM amino acids mixture and containing 0.5 μC of U-^{14}C-amino acid. Values are (dpm/mg protein) mean value ± SEM of 6 observations.

TABLE II

EFFECT OF INSULIN ON THE INCORPORATION OF U-^{14}C-LEUCINE AND U-^{14}C-PHENYLALANINE IN ISOLATED HEPATOCYTES CONTAINING LOW (FASTED 6-8 HRS) AND NO (FASTED 16-18 HRS) GLYCOGEN (dpm/mg protein)

		U-^{14}C-LEUCINE	U-^{14}C-PHENYLANANINE	U-^{14}C-LEUCINE	U-^{14}C-PHENYLANANINE
PERIOD OF INCUBATION		1 HR		3 HRS	
	GLYCOGEN LEVELS μmol glucose/g				
CONTROL	40* ± 20	960 ± 140	730 ± 80	2620 ± 180	1520 ± 130
CONTROL + INSULIN (100 μUnits)	40* ± 20	1180 ± 160	970 ± 100	3880 ± 210	1976 ± 140
CONTROL	NONE**	720 ± 90	620 ± 85	2130 ± 190	1380 ± 150
CONTROL + INSULIN (100 μUnits)	NONE**	680 ± 78	580 ± 70	2210 ± 180	1420 ± 160

Hepatocytes were prepared from 6-8* hr or 16-18** hr fasted rats as described previously (4). Approximately 55-75 mg of cells were incubated for 1-3 hrs in 3 ml of Umbreit Ringer Bicarbonate buffer (5) containing 7.5 mM glucose, 5 mM lactate, and 5 mM amino acids and containing 0.5 μC of U-^{14}C-amino acid. Values are (dpm/mg protein) mean values ± SEM of 4 observations.

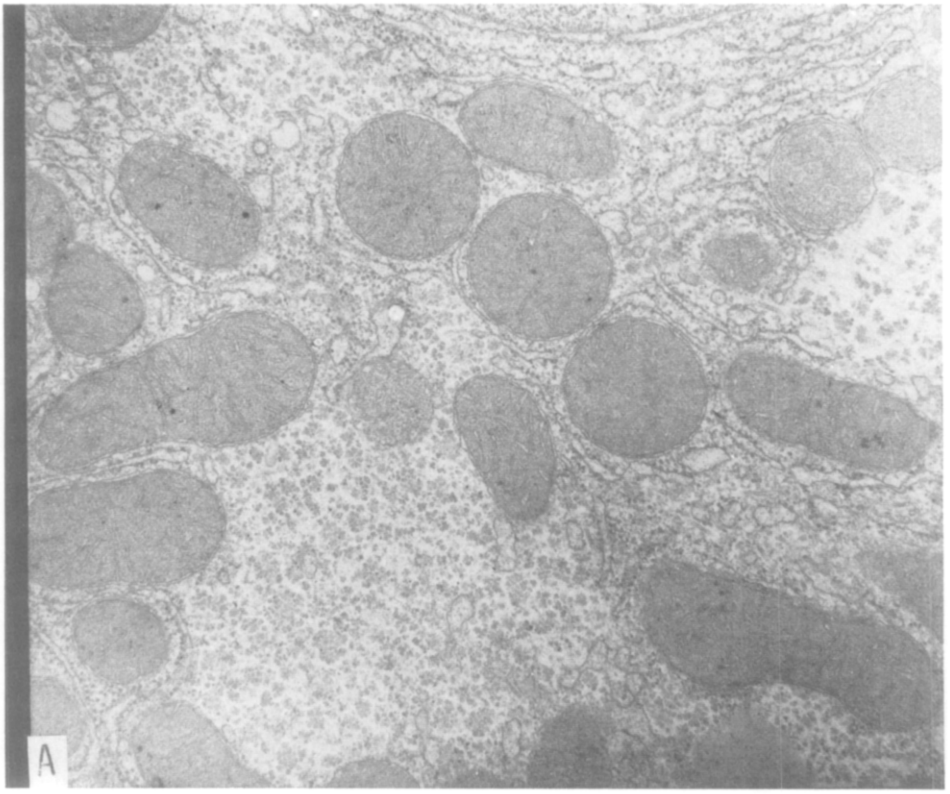

Figure 1-A: Electron micrograph (22,400 x mag) of isolated hepatocytes (control) incubated for one hour as described in Table I.

hepatocytes obtained from fasted rats or when glycogen content was low as is evidenced in Table II. Hepatocytes obtained from fasted rats (16-18 hrs) show a small decrease in the incorporation of amino acids into protein (Table II). This is in agreement with our previous observation (3) where a significant decrease in amino acids incorporation into protein was observed in hepatocytes obtained from 24 hr fasted animals. These studies suggest that the stimulatory effect of insulin on amino acid incorporation into protein is observed only in hepatocytes that contain high concentration of glycogen.

Studies on ultrastructure of incubated cells (1 hr) under various conditions are summarized in Figure I (A-D). Cells incubated with insulin showed

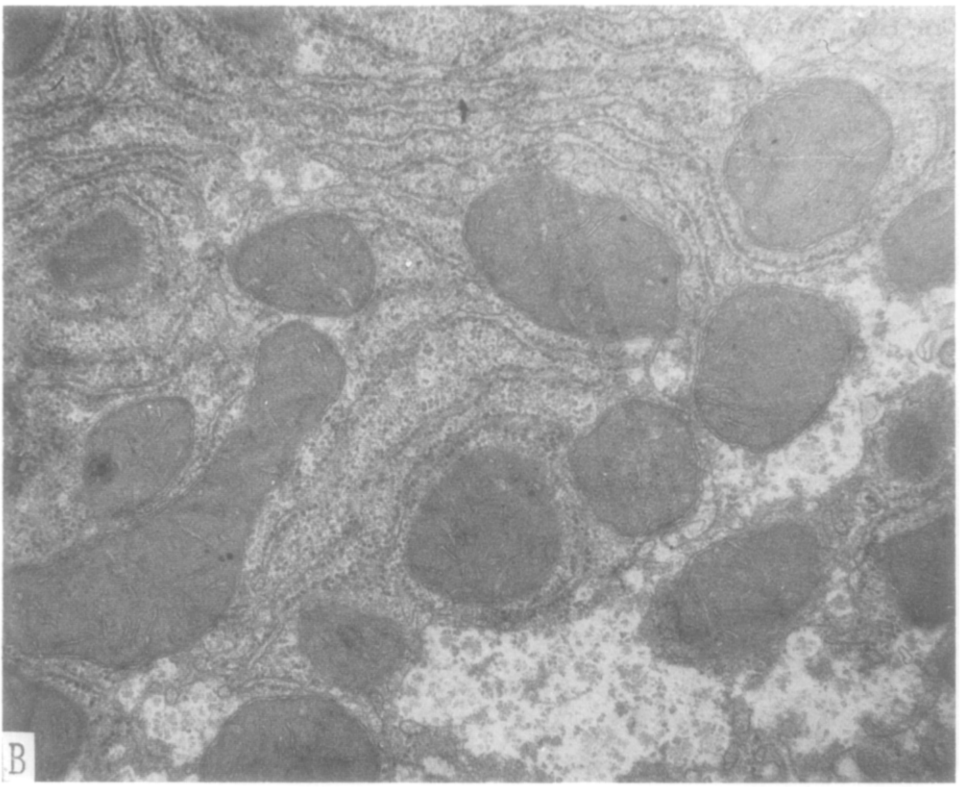

Figure I-B: Electron micrograph (22,400 x mag) of isolated hepatocytes [control + Insulin (50 μUnits)] incubated for 1 hour as described in Table I.

high intracellular glycogen, associated with more normal parallel strands of polyribosomes (Fig. 1-B) as compared with control (A), or glucagon (C), and glucagon and insulin treated cells (D). In the absence of insulin (Fig. 1-A) there is an increase in free ribosomes and a decrease in polysome strands and similar ultrastructural changes are also seen in the presence of glucagon (Fig. 1-C) and insulin and glucagon treated cells (Fig. 1-D). The results presented here suggest that insulin is involved directly both in glycogen and protein synthesis and that this synthesis may be mediated by a common messenger or

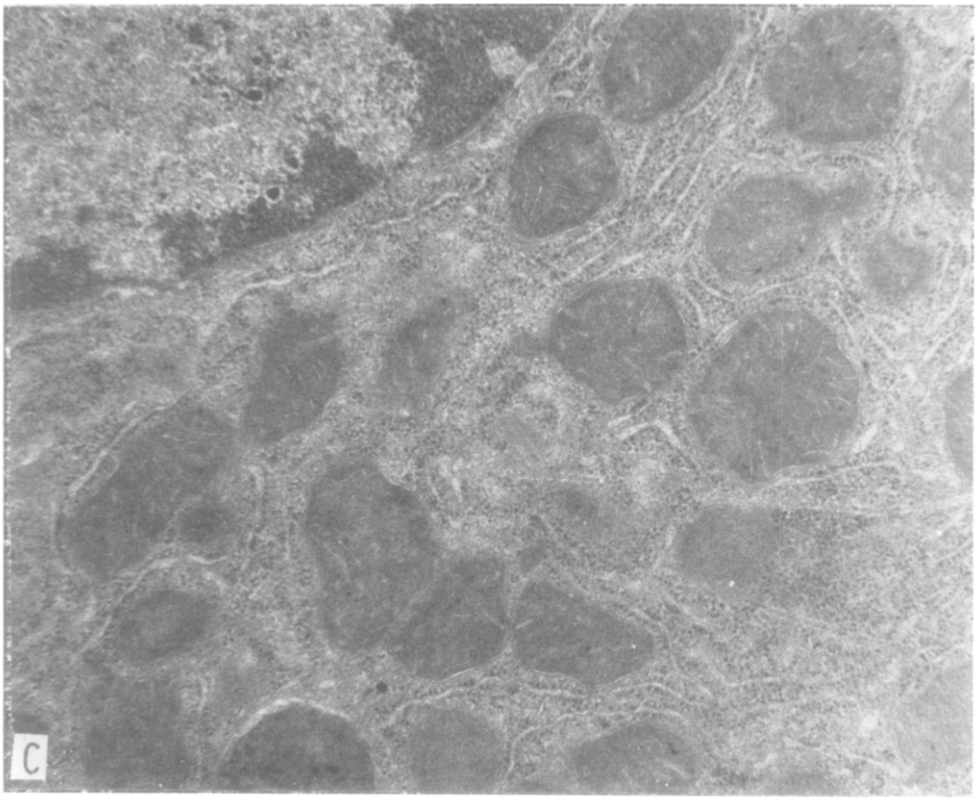

Figure 1-C: Electron micrograph (22,000 x mag) of isolated hepatocytes (control + glucagon 10^{-6} M) incubated for one hour as described in Table I.

that glycogen may act as the stabilizing factor to maintain polyribosome strands.

Crane and Miller (11) have shown that insulin and cortisol succinate both stimulated plasma protein synthesis by isolated hepatocytes. We have observed in our previous studies (12, 13) that insulin stimulated glycogen synthesis in isolated hepatocytes and also activated glycogen synthase by 16-40%. Pain et al. (14) have shown previously that administration of insulin to a diabetic rat resulted in reversion of rough endoplasmic reticulum to

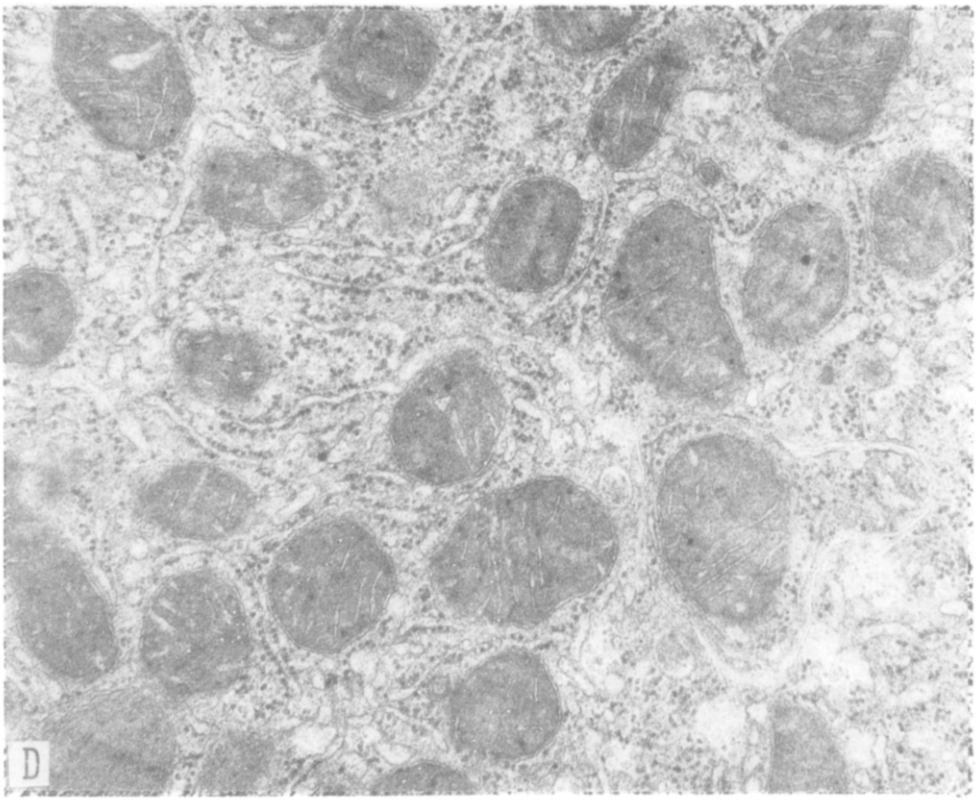

Figure I-D: Electron micrograph (22,400 x mag) of isolated hepatocytes (control + Insulin, 50 uUnits, + glucagon, 1 x 10^{-6} M) incubated for one hour as described in Table I.

parallel arrangement characteristic of the normal cell with a concommitant decrease in the amount of smooth endoplasmic reticulum and glycogen content was greatly increased. A decrease in amino acid incorporation into protein by isolated hepatocytes in insulin-deficient animals has been previously reported (15). The studies presented here show that addition of insulin to normal cells not only stimulates amino acid incorporation into protein but also helps to maintain normal parallel strands of polyribosomes and this effect requires high intracellular glycogen.

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REFERENCES

1. W.D. Lotspeich, J. BIol. Chem. 179:175, 1949.
2. D.M. Kipnis and G.F. Cori, J. Biol. Chem. 224:681, 1957.
3. S.R. Wagle, and W.R. Ingebretsen, Jr., Proc. Soc. Expt. Biol. and Med. 147:581, 1974.
4. W.R. Ingebretsen, Jr., and S.R. Wagle, Biochem. Biophys. Res. Comm. 46:403, 1972.
5. W.W. Umbreit, R.H. Burris and J.F. Stauffer, Monometric Technique P. 155, 1964.
6. S.R. Wagle, Biochem. Biophys. Res. Comm. 59:1366, 1974.
7. D. Monier, S. Santhaman and S.R. Wagle, Biochem. Biophys. Res. Comm. 46:1881, 1972.
8. C.A. Good, H. Kromer and M. Somagi, J. Biol. Chem. 100:485, 1933.
9. S.R. Wagle and J. Ashmore, J. Biol. Chem. 236:2868, 1961.
10. F.S. Reynolds, J. Cell. Biol. 17:208, 1963.
11. L.F. Crane and D.L. Miller, Biochem. Biophys. Res. Comm. 60:1269, 1974.
12. S.R. Wagle, W.R. Ingebretsen, Jr. and L. Sampson, Biochem. Biophys. Res. Comm. 53:937, 1973.
13. J. Akapan, R. Gardner and S.R. Wagle, Biochem. Biophys. Res. Comm. 61:22, 1974.
14. V.M. Pain, J. Lanoix, J.J.M. Bergerson and M.J. Clemens, Biochemica Biophysica Acta 353:487, 1974.
15. W.R. Ingebretsen, M.A. Moxley, D.O. Allen and S.R. Wagle, Biochem. Biophys. Res. Comm. 49:601, 1972.