as long as 3 days after challenge with the virus. The compound will inhibit further plaque formation even after cytopathic effects are already evident. Production of plaque-forming virus particles is likewise suppressed by the compound. These findings provide indirect evidence that varicella-zoster virus is a DNA virus, and they suggest that perhaps this compound might find some use in cases of serious human disease produced by this virus.

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Insulin Action and Protein Synthesis in Diaphragm Muscle.* (28849)

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The recognition of the role of nucleic acids in regulation of protein synthesis has directed attention to the possibility that the diverse effects produced by a hormone in responsive cell types might all be the result of a single primary action of a hormone at the genetic locus. This view, which may be designated as the hormone-gene thesis, first advanced in the case of the sex hormones(1,2), has been suggested for other hormones as well, since in certain cases characteristic "target" tissue responses are abolished by actinomycin D, known to inhibit DNA dependent RNA synthesis(3,4) and/or puromycin which blocks protein synthesis(5,6) without inhibition of RNA synthesis(7). Based upon studies with these inhibitors, the hormones whose action at a cellular level appears to involve stimulation of protein synthesis, presumably by way of the synthesis of messenger RNA, include estradiol(7,8), corticoids(9,10,11) and thyroid hormones(12,13). The recent finding of Wool(14) that insulin increases the synthesis of RNA in muscle raises the possibility that the multiplicity of effects of this hormone upon cell metabolism and membrane transport(15) might all be secondary to an effect of insulin at the genetic locus which leads to increased protein synthesis. To test this view, studies were undertaken to determine whether puromycin inhibits characteristic effects of insulin in rat diaphragm muscle. We wish to report here that concentrations of puromycin which almost completely abolish peptide bond synthesis do not influence insulin action upon either D-xylose transfer, α-aminoisobutyrate (AIB) transport or glycogen synthesis in the isolated intact rat diaphragm preparation.

Experimental. Experiments to determine the minimal concentration of puromycin which could effectively block glycine-C^{14} incorporation into diaphragm protein revealed that concentration of 0.11 mM puromycin

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TABLE I. Effect of Puromycin on Incorporation of Glycine-1-C^{14} into Protein of Rat Diaphragm at Varying Levels of Inhibitor and Substrate.

Puromycin, mM	Insulin	Glycine incorporation into proteins (mμM per g per 3 hr) Glycine concentration (mM) in medium .01	.2	.5	2.5
0	0	21.5 ± 2.8 (5)*	454 ± 29 (6)	855 ± 49 (5)	2450 ± 160 (4)
	+	75.0 ±15.0 (5)	1156 ± 69 (6)	1636 ± 59 (5)	5606 ± 273 (4)
.02	0	16.6 ± .4 (5)	190 ± 15 (5)	383 ± 15 (5)	
	+	27.4 ± .6 (5)	582 ± 31 (5)	881 ± 97 (5)	
.11	0			27 ± 5 (5)	136 ± 39 (4)
	+			45 ± 5 (5)	333 ± 62 (4)

* Mean ± standard error and number of observations. Intact rat diaphragm preparations incubated in 25 ml Krebs-Ringer bicarbonate (gas phase 95% O_2, 5% CO_2) containing glucose (2 mg/ml) for 3 hr with varying concentrations of glycine-1-C^{14} in presence and absence of insulin (0.5 U/ml). Muscle proteins were isolated and purified by a modified Schmidt-Tannhauser-Schneider procedure(16), estimated by the method of Lowry *et al.*(17), and the radioactivity counted in a Tri-Carb Scintillation counter to give specific activity (SA) values for protein. SA values were used to calculate incorporation of glycine-1-C^{14} into protein in terms of mμM glycine incorporated per 3-hr incubation per gram protein.

TABLE II. Lack of Effect of 0.11 mM Puromycin upon D-xylose and AIB Transport.

Puromycin, mM	Insulin	% distribution D-xylose	AIB
0	0	30 ± 3 (16)*	71 ± 8 (8)
	+	78 ± 2 (17)	235 ±23 (9)
.11	0	24 ± 2 (8)	96 ± 8 (8)
	+	82 ± 1 (8)	263 ±16 (8)

* Mean ± standard error and number of observations. Intact rat diaphragms were incubated in 25 ml Krebs-Ringer bicarbonate (gas phase 95% O_2, 5% CO_2) in presence and absence of insulin (0.5 U/ml) with and without puromycin. D-xylose distribution was determined after incubation for 60 min by methods previously described(18). Values given are calculated distribution in cell water using sucrose space as a measure of extracellular water. AIB distribution was determined after 90-min incubation using C^{14} labeled AIB and values expressed as a distribution in total tissue water (cpm/g tissue: cpm/ml plasma × 100)(19).

inhibited peptide-bond synthesis more than 90% (Table I) without influence upon the distribution of potassium or sodium. Addition of insulin to tissues treated with puromycin (0.11 mM) stimulated peptide bond synthesis, but the level achieved under these conditions is only about 5% of that obtained in the absence of added insulin in the case of control tissues. Table II shows the results of experiments on D-xylose and AIB transport, which illustrates that insulin retains its ability to increase membrane transport despite the presence of 0.11 mM puromycin. Table III shows that insulin retains its ability to stimulate glycogen synthesis in the presence of 0.11 mM puromycin. While insulin-induced incorporation of C^{14}-glucose into glycogen tends to be reduced in the presence of puromycin in relation to control as incubation time increases, it should be noted that the net synthesis of glycogen is not significantly inhibited by puromycin. It

TABLE III. Lack of Effect of Puromycin (0.11 mM) upon Insulin-Induced Glycogen Synthesis.

Puromycin, mM	Insulin	Glucose-C^{14} incorporation into glycogen, mg/g tissue (wet) 15′	45′	90′	Net glycogen synthesis, mg/g tissue (wet) 15′	45′	90′
0	0	.34 ±.1 (4)*	.32 ±.4 (4)	.31 ±.05 (9)			
	+	1.12 ±.1 (5)	3.24 ±.2 (4)	6.1 ±.3 (13)	1.2 ±.2 (8)	4.8 ±.2 (8)	7.9 ±.3 (9)
.11	0	.22 ±.1 (5)	.57 ±.4 (5)	.56 ±.1 (10)			
	+	1.14 ±.07(5)	2.78 ±.2 (9)	4.5 ±.4 (15)	1.8 ±.1 (8)	5.3 ±.3 (9)	7.5 ±.3 (8)

* Mean ± standard error and number of observations. Glucose-C^{14} incorporation into glycogen was determined by methods previously described(18). Net glycogen synthesis was determined by isolation of glycogen from KOH digests of muscle, followed by HCl hydrolysis and measurement of glucose equivalents using a glucose-oxidase method.

may be suggested that endogenous amino acids become available for glycogenesis as a result of puromycin treatment, with consequent dilution of the specific activity of the glycogen formed from glucose-1-C^{14}.

Discussion. The fact that well-established effects of insulin on membrane function and glycogen synthesis in diaphragm muscle can be achieved under conditions where peptide bond synthesis is almost completely abolished, demonstrates that these effects do not depend upon hormonal stimulation of protein synthesis. Although insulin does increase RNA synthesis(14), it would appear that the effect at the nuclear locus is secondary to a more primary effect of insulin. The question may be raised whether this may also be the case for hormones which influence protein synthesis, as based upon studies with actinomycin D and/or puromycin.

Summary. 1. The effect of puromycin upon glycine-1-C^{14} incorporation into the protein of the isolated intact rat diaphragm preparation was studied; it was found that 0.11 mM puromycin almost completely inhibits peptide bond synthesis in this system, both in presence and absence of added insulin. 2. In diaphragm preparations treated with 0.11 mM puromycin, insulin retains its ability to increase D-xylose transfer, AIB transport, and glycogen synthesis. 3. The effects of insulin to modify transport and metabolic processes in diaphragm muscle are not secondary to an effect of hormone at the nuclear locus to influence protein synthesis.

NOTE ADDED IN PROOF: While our paper was in press, the paper of D. Eboue-Bonis, A. M. Chambant P. Volfin and H. Clauser dealing with the action of insulin on isolated rat diaphragm in the presence of actinomycin D and puromycin appeared (*Nature*, 1963, v199, 1183). These authors studied the effect of inhibitors upon the ability of insulin to facilitate glucose uptake, biosynthesis of RNA and protein, and P^{32} labeling of "energy-rich" phosphate compounds. These authors report that concentrations of actinomycin D which completely inhibit labeling of RNA, do not influence the incorporation of labeled amino acids into protein, nor the influence of insulin to increase peptide-bond synthesis; labeling of ATP from adenine is likewise not effected by actinomycin D. Actinomycin or puromycin (in doses which completely inhibit amino acid incorporation into protein) do not inhibit the turnover of "energy-rich" phosphate compounds (*e.g.*, phosphocreatine, ATP, ADP, and uridine and guanosine phosphates), nor the effect of insulin to increase turnover rate. Finally, puromycin does not inhibit the effect of influence of insulin to enhance glucose uptake. They likewise conclude that the action of insulin does not appear to be explicable in terms of hormonal regulation of messenger RNA biosynthesis.

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