sensitivity of mice to experimental immune hemolytic anemia.

Materials and methods. The rabbit antimouse erythrocyte serum used in these studies was prepared and generously supplied to us by Dr. V. ADAMKIEWICZ. Its method of preparation and properties have been previously described[20]. The pooled antiserum had an agglutinating titer of 1024 agglutinin units per ml, as measured against fresh mouse erythrocytes[20]. The histamine-sensitizing factor (HSF) of *B. pertussis* was prepared as previously described[21]. This proteinaceous component of the bacillus is believed responsible, not only for the enhanced sensitivity to histamine observed in pertussis-inoculated mice, but also for other sensitizing effects of *B. pertussis*[1-3]. Propranolol (Inderal, Ayerst Labs) was generously supplied by Dr. SAHAGIAN-EDWARDS. CFW female mice, weighing between 14 and 20 g were obtained from Carworth Farms, New City, New York. 3 groups of 20 mice received i.p. injections of saline, 45 µg *N* of HSF (4 days before challenge), or 1 mg of propranolol (10 min before challenge). 2 additional control groups of 20 mice received HSF and propranolol only. The first 3 groups were then injected i.p. with 25 agglutinin units of antiserum. All 5 groups were then observed for 72 h for signs of toxicity and mortality, the latter being used as the end-point in determining any enhanced susceptibility to the experimental hemolytic state, as described by ADAMKIEWICZ et al.[20].

Effect of histamine-sensitizing factor (HSF) of *B. pertussis*, and propranolol on susceptibility of CFW mice to experimental immune hemolytic anemia

Sensitizing agent	Dose	Rabbit antimouse erythrocyte serum (agglutinin units)	Death rate
Saline	0.5 ml	25	1/20
HSF	45 µg N	25	18/20
Propranolol	1 mg	25	14/20
HSF	45 µg N	–	1/20
Propranolol	1 mg	–	0/20

All injections i.p. HSF injected 4 days prior to antiserum. Propranolol injected 10 min before antiserum. Deaths tabulated 72 h after antiserum challenge. Death rates from HSF plus antiserum: Chi-square for separate effects versus combined effect 43.3: $P < 0.001$. Death rates from propranolol plus antiserum: Chi-square for separate effects versus combined effect 32.4: $P < 0.001$.

Results and discussion. Very shortly after injection of the antiserum it was observed that the HSF- and propranolol-inoculated animals exhibited severe toxic symptoms. These symptoms included ruffling of the fur, tachypnea, prostration, and occasional convulsions. These signs were absent, or present to only a minor degree, in saline-injected controls receiving antiserum. The Table shows that 72 h after antiserum challenge the groups that had received antiserum alone, HSF alone or propranolol alone had death rates of only 1/20, 1/20, and 0/20, respectively. On the other hand, the groups that received HSF and antiserum or propranolol and antiserum had death rates of 18/20 and 14/20 respectively ($P < 0.001$). Thus there was clear potentiation of the lethal effect of the antiserum by both HSF and propranolol. A second experiment of like design produced similar results.

These results show that a component of *B. pertussis*, as well as the β-adrenergic blocking drug, propranolol, as had earlier been reported with insulin, are capable of enhancing the susceptibility of mice to experimental immune hemolytic anemia. Studies are currently underway to explore the mechanism(s) involved in the enhancing effect of the 3 agents on this hemolytic state, and to investigate further the role of blood and tissue glucose levels in the susceptibility of experimental animals to stressful stimuli[22].

Résumé. Un constituant de *Bordetella pertussis*, et la drogue antagoniste, le propranolol β-adrénergique, peuvent causer un renforcement semblable similaire de la sensibilité des souris à l'anémie hémolytique immune expérimentale. Il est connu que l'insuline se comporte de la même façon.

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A Probable Effect of Diabetes Mellitus on Incorporation of Amino Acids into Proteins Catalyzed by Isolated Rat Liver Nuclei

Whether hormones alter nuclear protein synthesis, believed to occur in vivo, is not certain, due to the flux of proteins across intracellular compartments[1-3]. The effect of alloxan-induced diabetes mellitus on the ability of isolated rat liver nuclei to catalyze incorporation of radioactive amino acids into hot TCA-insoluble materials (labeling of nuclear proteins) has been examined. Preparations from diabetic rats often exhibited reduced incorporation, which insulin in vivo could restore toward normal.

Methods. Male Sprague Dawley or Long Evans rats, rendered diabetic by i.v. injection of 50 mg of alloxan in 0.9% saline/kg body weight, were maintained on

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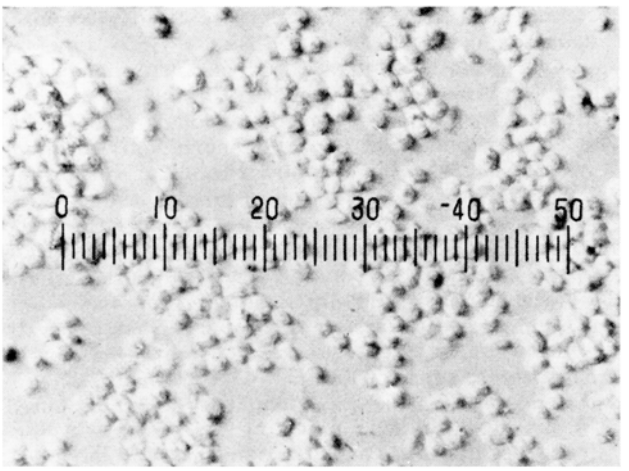

Phase contrast photomicrograph of isolated rat liver nuclei. Nuclei treated with detergent appeared similar. ×200.

Rockland mouse/rat diet and water ad libitum. Blood glucose was measured with Worthington 'Glucostat' kits or with Ames 'Dextrostix'. 3 days after alloxan, glucose concentrations exceeded 500 mg/100 ml, while 1–2 h after 5 units of regular insulin given i.p., they fell to 80–250 mg/100 ml, compared with the normal of around 115 mg/100 ml. Isolation of liver nuclei from control (N), diabetic (D), and insulin-treated diabetic (DI) rats, their purification in 2.2 *M* sucrose 1 m*M* $MgCl_2$ solution and washing with Triton-X-100, details of incubation and determination of radioactive protein followed published procedures[4–8].

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Table I. Effect of several agents on labeling of proteins catalyzed by isolated rat liver nuclei

Nuclear protein (mg/ml)	3.8		2.2		1.4	
	cpm/mg protein	% change	cpm/mg protein	% change	cpm/mg protein	% change
Experimental	95	–	326	–	272	–
RNase (1 mg/ml)	133	+40	318	— 2	313	+15
Cyanide (2.5×10^{-3} *M*)	10	—90	221	—32	–	–
Chloramphenicol (7.4×10^{-3} *M*)	–	–	140	—57	52	—81

^{14}C amino acid mixture or ^{14}C phenylalanine were incubated with nuclei in a sodium-containing system similar to those in the literature[6,7]. Gas flow (20% efficiency for ^{14}C) or liquid scintillation counting (58% and 20% efficiencies for ^{14}C and ^{3}H respectively) was used to measure radioactivity. Experimental values were usually 4–6 times the zero time controls.

Table II. Comparison of incorporation catalyzed by hepatic nuclei from normal (N), alloxan-diabetic (D), and insulin-treated (DI) rats

Experiment No.	Nuclear protein (mg/ml)	Label	Days post-alloxan	Nuclear protein (cpm/mg)		
				Normal	Alloxan-diabetic	Diabetic + Insulin
1.	1.05	1 μC/ala > 350 mc/m*M*	4	373 ± 19[a] (+14)	328 ± 18	592 ± 24 (+80)
CAP	1.05			86 (—77)	101 (—69)	130 (—78)
2.	1.0	0.5 μC a. a. mix. (≃ 220 mC/m*M*)	4	536 ± 23 (+38)	387 ± 20	521 ± 23 (+36)
3.	3.5	0.6 μC a. a. mix.	4	185 ± 14 (+64)	113 ± 10.5	136 ± 12 (+20)
4.	1.5	0.4 μC a. a. mix.	3	96 ± 9.8 (+32)	73 ± 8.6	
5.	3.2	1 μC a. a. mix.	3	340 ± 18.5 (+53)	222 ± 15	
6.	2.75	0.5 μC a. a. mix.	2		105 ± 10.3	188 ± 11 (+78)
7.	6	0.5 μC a. a. mix.	2		92 ± 9.6	110 ± 10.5 (+20)
Triton-X-100-washed nuclei[b]						
8.	1.34	DL-^{14}C-leucine (2–10 mC/m*M*)	3	26 ± 5.1 (+86)	14 ± 3.7	
9.	1.53	DL-^{14}C-leucine	3	16.7 ± 4.1 (+114)	7.8 ± 2.8	13.4 ± 3.7 (+72)
10.	1.35	L-^{14}C-glycine (2–10 mC/m*M*)	2	108 ± 10.3 (+50)	72 ± 8.5	
11.	1.56	L-^{3}H-leucine 5 C/m*M*	2	86 ± 9.3 (+43)	60 ± 7.7	71 ± 8.4 (+18)
12.	1.26	L-^{14}C-glycine	$1^1/_2$	58 ± 7.6 (+56)	37 ± 6.1	49 ± 7.0 (+32)

Insulin-treated rats received 5 units of regular insulin diluted with 0.9% saline; normal and diabetic animals received the same volume of saline. Rats were sacrificed $1\text{–}1^1/_2$ h later. In an individual experiment, incorporation catalyzed by equal amounts of nuclear protein from the 3 groups was compared. [a] One sample: Each result represents the average of 2 or 3 samples. [b] Nuclei incubated with from 1–4 μC of radioactivity. ± S.E.M. (), % change from diabetic control. CAP, Chloramphenicol, 7×10^{-3} *M*.

Results. Contamination of nuclei by non-nuclear elements was minimal, as judged by phase contrast and electron microscopy; detergent washed nuclei lacked the outer layer of the nuclear envelope with its cytoplasmic ribosomes (Figure, ref. [9]). Some characteristics of the incorporation observed with normal rat liver nuclei are presented in Table I; resistance to ribonuclease, and sensitivity to sodium cyanide and chloramphenicol have been used to distinguish this nuclear process from non-nuclear protein labeling[6,7]. To obtain this pattern, great care had to be taken to prepare highly purified nuclei, and inhibitor concentrations of $> 10^{-3} M$ were required.

Results obtained with liver nuclei from normal, alloxan-diabetic, and insulin-treated diabetic rats are presented in Table II. Nuclei from diabetic rats frequently exhibited reduced labeling, which in a number of experiments increased following insulin administration 1–1½ h before sacrifice. Whether this represents a true insulin-stimulated enhancement is not established: however, to reduce random variation, livers from 2 rats in each group were combined, and nuclei from the 3 groups isolated simultaneously. Both parenchymal and non-parenchymal cell nuclei might catalyze this incorporation.

Nuclei washed in Triton-X-100 also exhibited these changes, although greater variation was seen due to the use of 3 different isotopes. If specific radioactivity was expressed per unit of DNA, differences between (N) and (D) groups increased.

Discussion. In each experiment the level of incorporation must depend in a complex way upon the severity of the diabetes, concentration of nuclei, amount and specific radioactivity of the amino acid, etc. Before definitive conclusions about the effect of alloxan-induced diabetes mellitus on this process can be established, these interrelationships would have to be defined. If it is confirmed that insulin rapidly increases nuclear incorporation, an explanation for differences between (N) and (D) groups based on unspecified 'toxic' effects of alloxan will be rendered unlikely.

KORNER[10] found that 3 to 6 h after regular insulin, cell-free protein synthesis in hepatic microsomes from hypophysectomized rats increased 5 and 20% respectively; 1 h after insulin a decrease of 12% occurred. One week after alloxan administration rat liver polysome profiles did not change significantly, but 1 h after 10 units of regular insulin the abnormal profiles seen by the 18th day reverted to normal[11]. DOELL[12] observed an increased hepatic microsomal protein synthesis 12 h after insulin and glucose were administered to normal rats. An insulin-stimulated increase in hepatic nuclear protein synthesis preceding these reported effects on the cytoplasm is noteworthy.

Incorporation of radioactivity into nuclear proteins is related to the physiological activity of the cells of origin. GALLWITZ and MUELLER[13] observed that Hela cell nuclear protein synthesis increased shortly before cell division, while microsomal protein synthesis was reduced. 4–8 h after administration of *tri*-iodothyronine, TATA found that in vivo amino acid incorporation into hepatic nuclear proteins increased; nuclei isolated from hormone-treated rats first evidenced, increased in vitro incorporation several h later, but microsomes did not, unless isolation was delayed for at least 12 more h[14,15]. Rat uterus also exhibited stimulation of nuclear protein synthesis (estimated in vivo), which preceded the major estradiol-17β stimulation of microsomal protein synthesis[16].

It is interesting that such diverse agents as thyroxine, estradiol-17β, and insulin all seem to increase labeling of nuclear proteins before the major stimulation of cytoplasmic protein synthesis. These hormones can cause abrupt increases in target organ RNA synthesis; enhanced nuclear protein synthesis could be related to the formation of proteins associating with ribosomal or non-ribosomal RNA, and essential for their synthesis, protection from nucleases, transport to the cytoplasm, etc.[17,18]. The observation that within 2 h of insulin administration, the template activity of hepatic chromatin from diabetic rats reaches a peak, while insulin-induced glucokinase activity is not significantly increased at 3 h, is also germane[19].

The experimental difficulties presented by cell-free nuclear systems catalyzing the incorporation of amino acids into proteins are formidable. However, demonstration that such a process, stimulated before an effect on microsomes of a target organ, was partially hormone-dependent, could help to further define mechanisms of hormone action. It remains to be established whether the differences depicted in Table II were fortuitous or reflect a continuation in vitro of ongoing nuclear protein synthesis occurring in vivo. In pursuit of this problem, other approaches should not be overlooked[13,20–23].

Résumé. On a étudié l'incorporation des acides aminés radioactifs étiquettés dans des matériaux chauds, insolubles dans l'acide *tri*-chloroacétique (en marquant les protéines nucléaires), catalysés par des noyaux hépatiques extraits de rats normaux, de rats atteints de diabète causé par l'alloxane et de diabète traité à l'insuline. Dans plusieurs expériences, l'incorporation diminuée observée dans les noyaux provenant des rats diabétiques put être augmentée par l'insuline in vivo.

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