pletely removed and the 2267-cm^{-1} band was sharply reduced in intensity. These observations are in accordance with the known sensitivity of isocyanates to hydrolysis. The net result of reactions 1 and 2 is to provide a pathway to NH_3 in mixtures of NO, CO, and H_2O which does not involve molecular hydrogen, whether it is already in the stream or generated in situ over the catalyst by means of the water-gas shift reaction.

It is now known that Ru, of all the noble metals, is unique in its ability to remove NO from automobile exhaust without producing large amounts of ammonia (*2*). Reexamination of Fig. 1 will show that the isocyanate band is much weaker for Ru than for any of the other noble metals. Further, the isocyanate species on Ru could only be found on freshly prepared samples (see Table 1). These observations also correlate with the unique ability of Ru to switch between two metastable states one of which gives significant amounts of ammonia and one of which does not (*2*). The low ammonia-forming tendency of Ru can be due either to its ability to promote NO reduction without appreciable ammonia formation, or to its ability to promote facile decomposition of any ammonia formed. The observations (*2*) that "reduced" ruthenium gives low ammonia formation even at temperatures as low as 300° to 350°C whereas the same catalyst promotes appreciable ammonia decomposition only at temperatures above 400°C indicates that the ammonia decomposition route to low ammonia cannot be invoked under all conditions.

In reactor studies in our laboratory it has been observed that reaction mixtures containing NO, CO, and H_2O (no H_2) gave ammonia formation over a noble metal catalyst. Under similar conditions, a mixture containing CO and H_2O (no NO or H_2) gave no appreciable water-gas shift reaction over the same catalyst. Under these conditions, it seems possible that the observed ammonia formation may be due to the isocyanate mechanism. The situation in actual automobile exhaust is quite complex, and it is impossible from the spectral results alone to assess the relative contribution of the isocyanate mechanism to ammonia formation. Actual exhaust often contains both H_2 and CO, so that hydrogen reduction, CO reduction, and isocyanate mechanisms of NO reaction are competing. Moreover, nonequilibrium reaction conditions exist in a federal CVS (constant volume sampling) test, so that both the gas composition and the catalyst temperature are varying during a particular test and are, in addition, affected by parameters of the vehicle system that vary from one vehicle to the next. The results reported here indicate the existence of a previously unreported mechanism to ammonia formation over noble metal catalysts, and it will require additional work to determine the relative importance of this mechanism under the actual conditions encountered in a practical vehicle emission control system.

MARK L. UNLAND
Corporate Research Department,
Monsanto Company,
St. Louis, Missouri 63166

References and Notes

1. M. Shelef and H. S. Gandhi, *Ind. Eng. Chem. Prod. Res. Develop.* **11**, 2 (1972).
2. R. L. Klimisch and K. C. Taylor, paper presented at the spring meeting of the California Catalysis Society, San Francisco, 28–29 April 1972.
3. R. L. Klimisch and G. J. Barnes, *Environ. Sci. Technol.* **6**, 543 (1972).
4. J. B. Peri and R. B. Hannan, *J. Phys. Chem.* **64**, 1526 (1960).
5. J. Bugosh, R. L. Brown, J. R. McWhorter, G. W. Sears, R. J. Sippel, *Ind. Eng. Chem. Prod. Res. Develop.* **1**, 157 (1962).
6. Before each experiment the sample was heated in a vacuum to 400°C for 4 to 5 hours, treated with 100 torr of O_2 for 1.5 hours, evacuated for 20 minutes, treated with 100 torr of H_2 for 1 hour, and evacuated. The sample was then considered clean.
7. W. Beck and H. S. Smedal, *Angew. Chem. Int. Ed. Engl.* **5**, 253 (1966).
8. A. Maki and J. C. Desius, *J. Chem. Phys.* **31**, 772 (1959).
9. L. H. Jones, *ibid.* **27**, 468 (1957).
10. ———, *ibid.*, p. 665.

30 October 1972; revised 8 December 1972

Adenosine 3′,5′-Monophosphate in Pancreatic Islets: Glucose-Induced Insulin Release

Abstract. *Glucose-induced release of insulin from perifused rat islets is associated with elevated islet adenosine 3′,5′-monophosphate. If values for adenosine 3′,5′-monophosphate are compared to insulin release during theophylline or glucose stimulation and theophylline plus glucose stimulation, it suggests a minor role for adenosine 3′,5′-monophosphate in directly stimulating insulin release but a prominent role in modulating glucose-induced release of insulin.*

Intracellular regulatory phenomena in many endocrine systems are affected by adenosine 3′,5′-monophosphate (cyclic AMP) (*1*). Stimulation of insulin release by glucagon and inhibition by epinephrine are thought to be modulated by intracellular cyclic AMP (*2*). The following indirect evidence suggests that glucose-induced release of insulin also is in part influenced by perturbation of intracellular cyclic AMP. Infusion of cyclic AMP into the isolated perfused rat pancreas stimulates insulin release (*3*). Theophylline, a phosphodiesterase inhibitor, and glucagon, an adenyl cyclase stimulator, both elevate cyclic AMP and induce insulin release in the absence of glucose (*4*), and both potentiate glucose-induced release of insulin (*5, 6*). Imidazole, an agent that decreases intracellular cyclic AMP by stimulating phosphodiesterase, inhibits glucose-induced release of insulin (*7*). It has also been demonstrated that if the terminal mechanisms of insulin release are inhibited, theophylline will only partially restore this release (*8*), which indicates that cyclic AMP has its mechanism of action before the final step of insulin release. Finally, direct evidence of glucose-induced increases in intracellular cyclic AMP in static islet incubation systems has been negative (*2, 9*).

This report demonstrates elevated cyclic AMP during glucose stimulation in perifused rat islets, which suggests that cyclic AMP indeed may in part mediate glucose-induced release of insulin. Further observations suggest that cyclic AMP causes a synergistic action on this release.

Islets from fed Long-Evans male rats were isolated with collagenase and perifused at 37°C, according to the methods of Lacy (*10*). After a 35-minute stabilization period, stimulators were added and the perifusion was discontinued 2 or 20 minutes thereafter. Islets were removed from the perifusion chamber within 60 seconds and placed in boiling 1 m*M* theophylline for 10 minutes (*11*). The boiled extracts were centrifuged to remove particulate debris, lyophilized, and assayed in duplicate for cyclic AMP by a protein-binding radiodisplacement method (*12*). The perifusate was composed of 0.3 percent human serum albumin in phosphate-bicarbonate buffer (*p*H 7.4) containing 2.5 m*M* glucose, a concentration that does not stimulate phasic

insulin release. Perifusate was collected after a single passage through the islets at a rate of 1 ml/min, and immunoreactive insulin (IRI) was measured as previously described (*4*).

All data are reported as the mean value ± the standard error of the mean, (S.E.M.) and *P* values were determined by Student's *t*-test.

Patterns of insulin release and concentrations of cyclic AMP from perifused islets during stimulation and control periods are illustrated in Fig. 1. Figure 1A shows the characteristic first and second phases of insulin release during 20 minutes of 16.7 m*M* glucose stimulation. Total insulin release during glucose administration was 145 ± 16 ng/20 min per 200 islets versus the control of 15.9 ± 2.1 ($P < .001$), and islet cyclic AMP at the conclusion of the stimulatory period was elevated 2.5-fold. As shown in Fig. 1B, when glucose stimulation was terminated at 2 minutes, islet cyclic AMP was already elevated 2.5-fold. During a 20 minute theophylline stimulation (Fig. 1C), insulin release was monophasic and totaled 54.6 ± 9.8 ng/20 min per 200 islets versus the control of 15.9 ± 2.1 ($P < .01$). Cyclic AMP at the end of the theophylline stimulation was increased fourfold. Figure 1D illustrates the result when glucose and theophylline were added simultaneously; insulin release was 350 ± 90 ng/20 min per 200 islets versus the control of 15.9 ± 2.1 ($P < .001$), and islet cyclic AMP was elevated 5.5-fold.

Glucose-induced release of insulin has been characterized in man (*13*) and in vitro (*14*) by an early spike-type discharge (first phase) and a slower progressive and sustained discharge (second phase). This phenomenon is qualitatively similar in isolated perifused rat islets (Fig. 1a). Kinetic analyses of patterns of insulin release suggest that glucose mediates both phases of insulin secretion but by different mechanisms (*15*). A computerized model has been designed in which the first phase is thought to be a result of the rapid discharge of stored insulin from a small nonhomogeneous labile storage "compartment," and the second phase results from glucose-induced intracellular processes (Provision), providing insulin to the small compartment for release (*15*).

Since islet cyclic AMP is elevated during the first 2 minutes of glucose stimulation (Fig. 1b), cyclic AMP may be important for first-phase insulin release. Other possibilities are that elevated islet cyclic AMP measured during the first phase is related to initiation of the second phase, which begins at the onset of stimulation, or that glucose induces cyclic AMP elevation for reasons unrelated to insulin release.

Glucagon and theophylline (in the absence of stimulatory concentrations of glucose) have been demonstrated to augment islet cyclic AMP and cause a small second phase of insulin release (*6*) (Fig. 1c). Elevated islet cyclic AMP is also seen after 20 minutes of glucose stimulation (Fig. 1a). Comparing insulin release by 16.7 m*M* glucose (Fig. 1a) or 10 m*M* theophylline (Fig. 1c) illustrates that glucose is much more effective even at lower islet concentrations of cyclic AMP. Thus, insulin release directly caused by cyclic AMP appears to be of minor consequence. When theophylline was added to glucose (16.7 m*M*), total insulin release was increased to a greater degree than the added effect of either agent alone. This synergistic effect by theophylline suggests that cyclic AMP primarily modulates glucose-induced release of insulin. The above data suggest that glucose stimulates insulin release and may in addition modulate or amplify its own action by elevating cyclic AMP.

Since it appears that the etiology of diabetes mellitus is multifactorial, it is conceivable that some genetic defects proximal to the site of modulation of insulin release by cyclic AMP could be corrected by augmenting cyclic AMP. Conversely, defects distal to the modulatory sites would be more resistant to cyclic AMP elevations and thus theophylline analogs would have a limited effect on insulin release. In support of

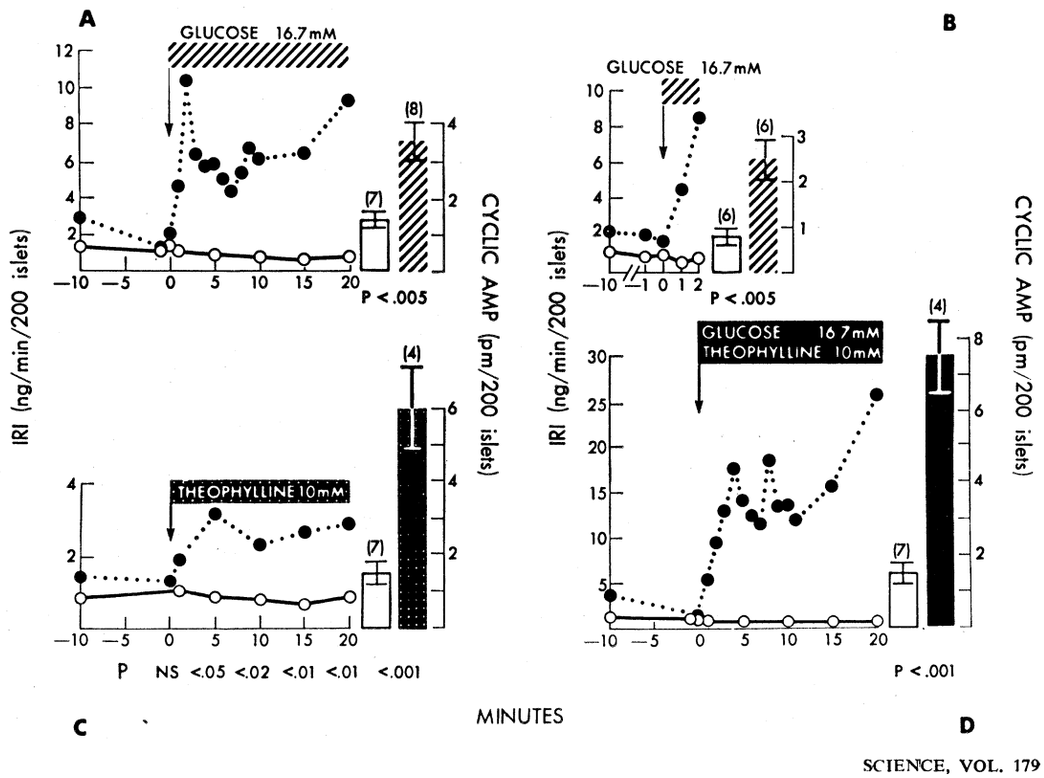

Fig. 1. Rat islet perifusion patterns and islet cyclic AMP during glucose-induced or theophylline-induced insulin release, or both. Each of the frames (A to D) represents perifused islet insulin release patterns on the left of each frame (each point represents the mean value), and islet cyclic AMP values are to the right as illustrated by the bar graphs (mean ± S.E.M.). Numerals in parentheses above bar graphs represent the number of experiments performed for both insulin release patterns (to left) and cyclic AMP determinations. *P* values under bar graphs represent significance of cyclic AMP determinations in controls versus stimulations. Note that ordinates in each frame are not of identical scale. Abbreviations: *pm*, picomole; *NS*, not significant.

this concept are the observations in "prediabetic" man (*16*) and in the isolated perfused pancreas of normal and diabetic Chinese hamsters (*17*) which indicate almost complete restoration of glucose-induced release of insulin by theophylline derivatives in some diabetics and no enhancement in others.

M. ARTHUR CHARLES
R. FANSKA, FLORENCE G. SCHMID
PETER H. FORSHAM
GEROLD M. GRODSKY
Metabolic Research Unit, Department of Biochemistry and Biophysics, University of California Medical Center, San Francisco 94122

References and Notes

1. A. G. Robison, R. W. Butcher, E. W. Sutherland, *Cyclic AMP* (Academic Press, New York, 1971), pp. 17–46.
2. J. R. Turtle and D. M. Kipnis, *Biochem. Biophys. Res. Commun.* **28**, 797 (1967).
3. K. E. Sussman and G. D. Vaughan, *Diabetes* **16**, 449 (1967).
4. G. M. Grodsky, L. L. Bennett, D. F. Smith, F. G. Schmid, *Metabolism* **16**, 22 (1967).
5. W. J. Malaisse, F. Malaisse-Lagae, D. Mayhew, *J. Clin. Invest.* **46**, 1724 (1967).
6. L. L. Bennett and G. M. Grodsky, in *Diabetes*, J. Ostman, Ed. (Excerpta Medica Foundation, Amsterdam, 1969), p. 462.
7. W. Malaisse, F. Malaisse-Lagae, S. King, *Diabetologia* **4**, 370 (1968).
8. G. R. Brisson, F. Malaisse-Lagae, W. J. Malaisse, *J. Clin. Invest.* **51**, 232 (1972).
9. W. Montague and J. R. Cook, *Biochem. J.* **122**, 115 (1971).
10. P. E. Lacy, M. M. Walker, C. J. Fink, *Diabetes* **21**, 987 (1972). We thank Dr. Paul Lacy for his interest and encouragement, enabling us to establish a perifusion system.
11. Results with islets homogenized in 5 percent trichloroacetic acid followed by acid-ether extraction and lyophilization were comparable to islets boiled in theophylline.
12. A. G. Gilman, *Proc. Nat. Acad. Sci. U.S.A.* **67**, 305 (1970).
13. S. Porte, *J. Clin. Invest.* **48**, 2309 (1969).
14. G. M. Grodsky, L. L. Bennett, D. Smith, K. Nemechek, in *Tolbutamide after Ten Years*, W. J. H. Butterfield and W. Van Westering, Eds. (Excerpta Medica Foundation, Amsterdam, 1967), p. 11.
15. G. M. Grodsky, *J. Clin. Invest.* **51**, 2047 (1972).
16. E. Cerasi and R. Luft, *Horm. Metab. Res.* **1**, 162 (1969).
17. B. Frankel and G. M. Grodsky, in preparation.
18. We thank Drs. M. Mozen and A. Pappenhagen of Cutter Laboratories, Berkeley, Calif., and Dr. J. Holper of Abbott Laboratories, South Pasadena, Calif., for their generous assistance in providing the supplies of albumin necessary for these studies. This study was supported in part by National Institute of Allergy and Metabolic Diseases grant AM-01410 and by the Hoechst Pharmaceutical Company, Somerville, N.J.; the Levi J. & Mary Skaggs Foundation; and the Kroc Foundation. M.A.C. is a postdoctoral fellow of the U.S. Army.

20 October 1972

Automatic Screening of Biological Specimens by Optical Correlation

Abstract. *An optical analog correlation technique was used to detect morphologic abnormalities in rat liver cells. The method employs an optical matched filter for correlating a test pathological specimen with a control specimen. Experimental results show appreciable promise that such an optical correlator can be employed as a tool for rapid mass screening of a variety of pathological specimens.*

The automation of a number of laboratory procedures relevant to the study of cells and their internal structures has been successfully accomplished. There are presently available automated methods for counting erythrocytes and leukocytes, detecting circulating neoplastic cells, and differentiating normal and neoplastic cells in cytological preparations. These automated techniques are often complex and time consuming, and most require an on-line digital computer for data storage and handling. In recent years, particularly since the advent of continuous wave lasers, optical data processing techniques have found many applications in engineering (*1*) and biological fields (*2*).

The technique presented in this report performs a real time correlation between a microscopic specimen of known pathology and one of unknown pathology from the same organ of a different individual. A technique utilizing a multitude of samples (as in raster scanning) would not produce real time correlation since each sample must be stored in a memory bank and the correlation performed only after sufficient data points to represent the entire specimen have been stored. A raster of sufficient resolution to analyze this specimen would divide the specimen into approximately 2000 scan lines each consisting of 2000 points with intensities relating to the intensities of the points in the original specimen. A large area or an entire section of the organ can be instantaneously inspected at one time, which eliminates the need for raster scanning of the specimen. The maximum area of inspection can be as large as a standard microscope slide. The possibility of automatic screening of biological specimens by an optical character recognition process stems from the fact that cells and tissue often react to disease or injury by undergoing morphological changes which are similar for a particular pathogen or injury in a particular organ. Examples of morphological changes are cellular swelling, cytoplasmic and nuclear degeneration, and cytokinesis (cytoplasmic changes during cell division). This cytomorphosis produces significant changes in the light scattering characteristics of the specimen. The optical matched filtering technique permits high signal-to-noise ratio detection of such morphological changes. The process in its present state of development is essentially a generalized formulation of the Weiner optimum filtering theory (*3*), which is easily realized in optical data processing.

The methodologies of coherent optical correlation are well documented (*4*). The experimental arrangement used in the study reported here is shown schematically in Fig. 1. A microscope slide containing a normal rat liver specimen, prepared by a standard hematoxylin and eosin staining process, is placed in the input plane (x, y). The function $f(x, y)$ denotes the amplitude transmission function of the object when it is illuminated by a collimated beam of coherent light. The lens L_1 focuses the two-dimensional

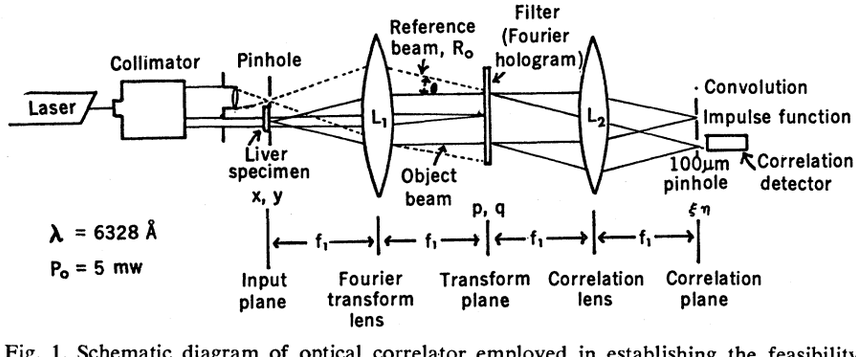

Fig. 1. Schematic diagram of optical correlator employed in establishing the feasibility of automatic detection of morphologically abnormal rat livers. The reference beam, represented by the dashed lines, is blocked off during the correlation measurements.