2650-Å wavelength on enzymes, as well as the theoretical effect on nucleic acids. In addition, the short pulse duration and high energy densities of this and more advanced laser systems are opening up several areas to the photochemist: studies on transient changes, biphotonic processes, intersystem crossing to the triplet state, and possibly even studies on the lifetimes of excited states of proteins and nucleic acids.

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References and Notes

1. M. W. Berns and D. E. Rounds, *Ann. N.Y. Acad. Sci.* **168**, 550 (1970).
2. ———, *Sci. Amer.* **222**, No. 2, 98 (1970).
3. J. M. Ingelman, T. C. Rotte, E. Schecter, D. J. Blaney, *Ann. N.Y. Acad. Sci.* **122**, 790 (1965).
4. D. E. Rounds, *Fed. Proc.* **24**, Suppl. 14, 116 (1965).
5. F. M. Johnson, *Nature* **204**, 985 (1964).
6. G. J. Fisher, J. C. LeBlanc, H. E. Johns, *Photochem. Photobiol.* **6**, 757 (1967).
7. H. Laser, in *Radiation Effects in Physics, Chemistry, and Biology*, M. Ebert and A. Howard, Eds. (Year Book, Chicago, 1963), p. 439.
8. Supported by the U.S. Army Research and Development Command, Office of the Surgeon General, under contract DA-49-MD-2564. We acknowledge Dr. J. K. Thomas of the Argonne National Laboratory for the sample of malachite green. The lactate dehydrogenase was grade A, purchased from Calbiochem Co., Los Angeles, Calif.

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Polypeptide with Broad Biological Activity: Isolation from Small Intestine

Abstract. *A polypeptide, which has potent and diverse biological action—including systemic vasodilation, hypotension, increased cardiac output, respiratory stimulation, and hyperglycemia—was isolated from the small intestine of the hog. The peptide has 28 amino acid residues and is chemically distinct from the kinins, "substance P," glucagon, and secretin.*

Polypeptide hormones regulate many physiologic functions and mediate certain pathologic responses. We report here the isolation of a new polypeptide possessing an unusually wide range of biological activity affecting cardiovascular, respiratory, and metabolic functions. The peptide, extractable from small intestine, causes systemic vasodilation, hypotension, increased cardiac output, stimulation of respiratory chemoreceptors, and hyperglycemia. Chemically, it has an amino acid composition that distinguishes it from other naturally occurring peptides with similar actions, such as the kinins, "substance P," and glucagon.

The peptide was prepared from the starting material (methanol extract of hog small intestine) used by Jorpes and Mutt for the preparation of secretin (*1*). The purification procedures consisted of two steps of ion-exchange chromatography, counter-current distribution, and gel chromatography. On chromatography on carboxymethyl cellulose in 0.0125*M* phosphate buffer, the active fraction was retained on the column but eluted with 0.2*M* HCl. It was chromatographed again on carboxymethyl cellulose in 0.1*M* ammonium bicarbonate. The active material emerged from the column late but with the same buffer. It was then subjected to counter-current distribution in a system of 1-butanol and 0.1*M* NH_4HCO_3. After a 200-tube transfer, the material in tubes 60 to 85 was recovered and a portion of it was hydrolyzed and analyzed for amino acids by two-dimensional paper chromatography (*2*). Two amino acids, glycine and proline, were present in much smaller amounts than any of the other amino acids found. The bulk of the material was then chromatographed in 0.2*M* acetic acid on Sephadex G-25 (Pharmacia). On hydrolysis and quantitative amino acid analysis (*3*), the active fraction was found to contain no proline and only a trace of glycine (less than one-tenth, on a molar basis, that of any other amino acid present). Tryptophan and cysteine/cystine were absent, as determined by the Voisnet-Rhode dimethylaminobenzaldehyde reaction (*4*) and by quantitative analysis of material that had been oxidized with performic acid before acid hydrolysis (*5*), respectively. The quantitative amino acid analysis suggested a polypeptide with 28 residues, having the following composition: 2 alanine, 5 aspartate/asparagine, 2 arginine, 1 glutamate/glutamine, 1 histidine, 1 isoleucine, 3 leucine, 3 lysine, 1 methionine, 1 phenylalanine, 2 serine, 2 threonine, 2 tyrosine, and 2 valine.

All ratios for the molar quantities of the amino acids were reasonably near whole numbers, indicating that the polypeptide was in an essentially pure form. This was supported by the appearance of one band on electrophoresis in polyacrylamide gel (*6*) and the finding of only one *N*-terminal amino acid, histidine, by the Edman method (*7*).

The absence of glycine and proline residues distinguishes this newly isolated peptide from other vasoactive peptides, the kinins (*8*) and "substance P" (*9*). Like secretin (*10*) and glucagon (*11*), the new peptide has an *N*-terminal histidine residue, but the lack of glycine and the presence of isoleucine clearly set it apart from these two hormones.

Investigation of the biological effects of the peptide was carried out in dogs anesthetized with pentobarbital or with chloralose and urethane. Blood flow in ascending aorta (cardiac output) and other systemic arteries was measured by noncannulating electromagnetic flow probes and flowmeter (Carolina Medical Electronics). Arterial blood pressure was recorded by Sanborn 267B transducers connected to an intra-aortic catheter, and respiratory minute volume was monitored by electronic integration of the signal from a pneumotachygraph attached to the airway. The concentration of glucose in the blood was estimated by a hexokinase-coupled enzymatic method.

Bioassay was based on the systemic vasodilator action reported for partially purified fractions (*12*). Assays were repeated in the same animal and in numerous animals. Intra-arterial infusion of the pure peptide at doses of 40 ng/kg increased femoral arterial flow by 50 percent. Larger doses (400 ng/kg) tripled blood flow and kept it above normal for 27 minutes. Local injection also increased superior mesenteric arterial flow (34 percent), but renal arterial flow did not change. On intravenous infusion of the latter dose, mean systemic arterial blood pressure fell by 15 mm-Hg, and total cardiac output increased by 43 percent ($P < .001$). The increase in cardiac output was due more to a greater (29 percent) stroke volume than to a faster (8 percent) heart rate. A possible direct myocardial action has not been established.

Respiratory minute volume was augmented by 30 percent, the hyperpnea resulting both from an increased frequency and a larger tidal volume. To determine the mechanism of this hyperventilation, we infused the peptide into one common carotid artery before and

after section of the carotid sinus nerve or inactivation of the carotid chemoreceptors by 3*M* acetic acid (*13*). Respiratory stimulation, amounting to a 49 percent increase in minute ventilation, was nearly abolished after chemoreceptor denervation.

The concentration of glucose in the blood, measured 15 minutes after intravenous infusions of 1 μg of the peptide per kilogram, increased by about 28 mg per 100 ml ($P < .001$). This increase was approximately one-third the corresponding rise following an equal dose of glucagon (Eli Lilly & Co.) in the same animals.

The diverse and potent biologic effects of this newly isolated peptide suggest possible physiologic roles, particularly in the control of intestinal blood flow and blood sugar. Its hyperglycemic action raises the possibility that it might be related to one of the glucagon-like factors demonstrated in extracts of small intestine (*14*). Since the peptide appears to be inactivated principally in the liver (*12*), it is unlikely that its actions normally extend beyond the splanchnic circulation. The peptide could, however, play an important pathogenetic role in situations where it is excessively released—for example, intestinal ischemia (*15*)—or inadequately removed—for example, hepatic cirrhosis. The changes described here are similar to those in hepatic cirrhosis (*16*)—increased cardiac output, peripheral vasodilation, relative impairment of renal blood flow, and abnormal glucose tolerance.

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References and Notes

1. J. E. Jorpes and V. Mutt, in *Ciba Foundation Symposium on the Exocrine Pancreas* (Churchill, London, 1962), p. 150.
2. R. R. Redfield, *Biochim. Biophys. Acta* **10**, 344 (1953).
3. D. H. Spackman, W. H. Stein, S. Moore, *Anal. Chem.* **30**, 1190 (1958).
4. J. R. Spies and D. C. Chambers, *ibid.* **21**, 1249 (1949).
5. S. Moore, *J. Biol. Chem.* **238**, 235 (1963).
6. E. W. Johns, *Biochem. J.* **104**, 78 (1967).
7. B. Blombäck, M. Blombäck, P. Edman, B. Hessel, *Biochim. Biophys. Acta* **115**, 371 (1966).
8. M. Schachter, *Fed. Proc.* **27**, 49 (1968).
9. M. M. Chang and S. E. Leeman, *ibid.* **29**, 282 (1970).
10. J. E. Jorpes, V. Mutt, S. Magnusson, B. B. Steele, *Biochem. Biophys. Res. Commun.* **9**, 275 (1962).
11. W. W. Bromer, L. G. Sinn, O. K. Behrens, *J. Amer. Chem. Soc.* **79**, 2807 (1957).
12. S. I. Said and V. Mutt, *Nature* **225**, 863 (1970); P. J. Piper, S. I. Said, J. R. Vane, *ibid.*, p. 1144.
13. H. A. Kontos, G. W. Vetrovec, D. W. Richardson, *J. Appl. Physiol.* **28**, 561 (1970).
14. E. Samols, J. Tyler, G. Marri, V. Marks, *Lancet* **1965-II**, 1257 (1965); A. M. Lawrence *Proc. Nat. Acad. Sci. U.S.* **55**, 316 (1966); R. H. Unger, A. Ohneda, I. Valverde, A. M. Eisentraut, J. Exton, *J. Clin. Invest.* **47**, 48 (1968); I. Valverde, D. Rigopoulou, J. Exton, A. Ohneda, A. Eisentraut, R. H. Unger, *Amer. J. Med. Sci.* **255**, 415 (1968).
15. E. E. Selkurt, *Amer. J. Physiol.* **197**, 281 (1959).
16. F. E. Tristani and J. N. Cohn, *J. Clin. Invest.* **46**, 1894 (1967); P. Vanamee, J. W. Poppell, A. S. Glicksman, H. T. Randall, K. E. Roberts, *Arch. Int. Med.* **97**, 762 (1956); J. R. Collins and O. B. Crofford, *ibid.* **124**, 142 (1969).
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Coliform Aerosols Emitted by Sewage Treatment Plants

Abstract. *Development of the science of aerobiology has furnished a tool for the investigation of potential sources of microbial aerosols. An investigation of aerosols emitted by trickling-filter sewage treatment plants revealed that coliforms were indeed emitted and have been sampled to a distance of 0.8 mile (1.2 kilometers) downwind. Factors affecting survival of* Escherichia coli *are presented.*

The association of pathogenic microorganisms with water and sewage has been known since 1855 when John Snow in London traced the source of a cholera epidemic to a sewage-contaminated well (*1*). Since that time human fecal waste has been found to contain the specific etiologic agents of some diseases, many of which are intestinal diseases. Although these are commonly transmitted through the mouth, experimental infection of the chimpanzee by inhalation of large numbers of aerosolized typhoid organisms has been demonstrated (*2*). However, there are other organisms, whose respiratory dosage is comparatively low, which are excreted in the fecal waste of infected persons. Some of these are: various respiratory viruses and the microorganisms that cause brucellosis, encephalitis, hepatitis, poliomyelitis, psittacosis, and tuberculosis.

The development of the science of aerobiology in the last few years provided a tool that has encouraged us to investigate potential sources of aerosolized microorganisms. Schultze (*3*), in 1943, studied the fallout of small droplets resulting from watering crops with liquid raw sewage from an overhead sprinkling irrigation system in Germany. Using a primitive sampling technique, he placed open petri dishes at varying distances downwind from the sprinklers and was able to demonstrate the presence of *Escherichia coli* in the airborne droplets. Spendlove (*4*), in 1956, demonstrated the aerosolization of bacteria from a rendering plant and was able to recover airborne organisms downwind from the plant with the use of Andersen samplers.

Modern trickling-filter sewage treatment plants, because of the nature of their design, may be an exceptional source of aerosolized microorganisms. As we contemplated the spectrum of potential aerosols, it became plausible that the variety of organisms that may be aerosolized is almost unlimited. The trickling filter used in the secondary treatment of sewage sprinkles raw sewage into the open air onto a rock ballast to dose the filter bed. The process of sprinkling the raw sewage into the air would be expected to aerosolize a portion of the material and create micron-size particles (Fig. 1). Sewage varies considerably in its microbial count, but counts of from 10^6 to 10^7 organisms per milliliter are common (*5*). A sewage plant processing several million gallons of sewage per day has the potential, therefore, of providing a microbial aerosol source of considerable magnitude on a continuous basis.

Two municipal sewage plants, ranging in treatment capacity from 6 to 25 million gallons (1 gallon = 3.7 liters) of sewage per day, were studied. The plants were located in the Intermountain West and the studies were conducted during May 1970. Andersen samplers (*6, 7*), connected to a portable field vacuum source, were used to collect the aerosols near and downwind of sewage treatment plants. The Andersen sampler aspirates at the rate of 1 cubic foot (28.3 liters) per minute and impinges the collected organisms on a nutrient medium placed in petri plates positioned within stages of the sam-