to the search for similar effects with other neoplasms and with a broader spectrum of chemotherapeutic compounds.

T. F. GALLAGHER, ATTALLAH KAPPAS, HERTA SPENCER, DANIEL LASZLO
Sloan-Kettering Institute for Cancer Research and *Montefiore Hospital, New York*

References and Notes

1. This investigation was supported by a grant from the American Cancer Society and by research grants (C-322 and C-1540) from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, Bethesda, Md.
2. R. Hertz, *et al.*, *Recent Progr. Hormone Research* 11, 119 (1955).
3. The methods employed have been described by A. Kappas and T. F. Gallagher, *J. Clin. Invest.* 34, 1566 (1955), and earlier publications.
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Partial Inactivation of Lipoprotein Lipase by Bacterial "Heparinase"

Several independent lines of evidence have indicated the existence of a rather close relationship between lipoprotein lipase and heparin. Indeed, the discovery of the enzyme derived from the fact that, upon the injection of heparin, the lipase appears in the blood, where its presence is visibly demonstrable in lipemic animals (*1*). The direct isolation of lipoprotein lipase from tissues (heart and adipose) of normal animals has been reported previously (*2*). In such preparations, it is possible to demonstrate an activation of the enzyme by added heparin. Lipoprotein lipase is inhibited by protamine. It can also be inhibited by pyrophosphate, and this inhibition, at least in the preparations from rat heart, can be reversed by heparin. More recently, Robinson (*3*) has reported that the heat stability of lipoprotein lipase of postheparin plasma is decreased by passing the plasma over an anion exchange resin; the addition of free heparin to such a preparation restabilizes the enzyme.

The isolation in this laboratory of a *Flavobacterium* (*4*) that is able to use heparin as its sole source of carbon, nitrogen, and sulfur, and the preparation of bacterial extracts (*5*) that possess "heparinase" (*6*) activity have provided another tool for the study of the interrelationship between heparin and lipoprotein lipase.

The data summarized in Table 1 show the results obtained on the incubation of lipoprotein lipase with the bacterial extract. Acetone powders of adapted bacteria were extracted with water and dialyzed against 0.005*M* ethylenediaminetetraacetate and then against water overnight. The precipitated protein was dissolved in 0.025*M* glycylglycine buffer at *p*H 7.5 and diluted to a protein concentration of 1 mg/ml. The lipoprotein lipase was a purified preparation from chicken adipose tissue (*7*) dissolved in 0.025*M* NH_3 to a protein concentration of 5 mg/ml. The bacterial extract and lipoprotein lipase (0.2 ml) were preincubated together in a total volume of 0.4 ml of 0.025*M* glycylglycine which contained 0.005*M* $MgCl_2$ for 15 minutes at 24°C. In each instance, the proper control, without the bacterial extract, was run. The vessels were then heated at 40°C for 5 minutes to inactivate selectively the heparinase. Four milliliters of 10-percent albumin at *p*H 8.5, 0.05 ml of 1*M* ammonium sulfate, and 0.05 ml of 2-percent activated coconut oil (*8*) were added to all the vessels, which were then incubated at 37°C for 1 hour for the determination of lipoprotein lipase activity (*2*). The glycerol that was produced was oxidized by periodate to formaldehyde, which was then determined colorimetrically with chromotropic acid.

Under optimum conditions, the lipase can be 60 percent inactivated. The component of the bacterial extract responsible for this inactivation is a nondialyzable, heat labile substance that exhibits all of the known properties of the heparinase (*5*). These are (i) complete inactivation when heated for 5 minutes at 40°C, (ii) a requirement for one of several ions for activity (magnesium, ammonium, phosphate, arsenate, or citrate), and (iii) inactivation when incubated in the presence of 0.2*M* NaCl or phosphate buffer. The bacterial extracts have some slight proteolytic activity, but this shows none of the afore-mentioned characteristics. Also, extracts of acetone powders of bacteria that had not been adapted to heparin do not inactivate lipoprotein lipase even though they possess equal proteolytic activity. On the basis of this evidence, it is concluded that the heparinase is responsible for the inactivation of the lipase and that lipoprotein lipase is, most probably, a mucoprotein which contains a mucopolysaccharide very similar to heparin as an integral part of the molecule.

Table 1. Inactivation of lipoprotein lipase by bacterial heparinase.

Additions during preincubation	Glycerol production (D_{570})
None	0.215
Bacterial extract, 0.01 ml	0.120
Bacterial extract, 0.10 ml	0.080
Bacterial extract, 0.01 ml + 0.2*M* NaCl	0.220
Heated* bacterial extract, 0.01 ml	0.210
Bacterial extract, 0.10 ml; no Mg^{++}	0.155

* Heated at 40°C for 5 minutes.

EDWARD D. KORN
Laboratory of Cellular Physiology and Metabolism, National Heart Institute, National Institutes of Health, Bethesda, Maryland

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4. A. N. Payza and E. D. Korn, *Nature* 174, 88 (1956).
5. E. D. Korn and A. N. Payza, *Biochim. et Biophys. Acta* 20, 596 (1956).
6. The term *heparinase* is used to refer to a group of at least three enzymes (*5*) that catalyze an extensive degradation of heparin.
7. E. D. Korn and T. W. Quigley, unpublished data.
8. Activated coconut oil is prepared by incubating a commercial coconut-oil emulsion with the α-lipoprotein fraction of normal plasma. The oil is then washed repeatedly by centrifugation through 0.15*M* NaCl. This washed, activated coconut oil contains a small amount of protein, cholesterol, and phospholipid, and it is identical to chylomicrons with respect to the action of lipoprotein lipase.

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Man is to himself the most wonderful object in nature; for he cannot conceive what the body is, still less what the mind is, and least of all how a body should be united to a mind. This is the consummation of his difficulties, and yet it is his very being.—B. PASCAL, *Pensées.*