specific activity of 7×10^5 units per milligram of protein. In addition, the sheep plasma erythropoietin preparation used for the experiment shown in Table 3 had a specific activity of 5000 units per milligram of protein (56 percent pure) (*10*), compared with 200 units per milligram of protein for the preparation used in all other experiments reported here.

Several observations make it unlikely that the suppressions of erythropoietin-stimulated hemoglobin synthesis and CSF-mediated colony formation were due to nonspecific actions of the impure preparations of CSF and erythropoietin: (i) CSF suppressed the action of erythropoietin, and erythropoietin that of CSF, at physiologic concentrations (approximately $10^{-11}M$); (ii) heat inactivation of the CSF preparation completely abolished its capacity to suppress erythropoietin action (Table 2); and (iii) CSF alone had no effect on baseline hemoglobin synthesis by marrow cells.

In contrast to our findings, Mitchell and Adamson (*11*) observed no reduction in the response by rat marrow cells to erythropoietin after incubation of the cells with CSF. A possible explanation for this difference may be found in the source and purity of the CSF. Mitchell and Adamson used a relatively crude material prepared from rat leukocyte culture medium. We have found that unfractionated serum from rats and mice, injected with endotoxin 3 hours prior to bleeding, is a potent stimulator of granulocyte-macrophage colony formation, but does not suppress the response by marrow cells to erythropoietin. In contrast, both human urinary CSF and CSF prepared from L cell culture medium are effective suppressors of erythropoietin action.

The results of our studies in vitro provide support for what has been found repeatedly in vivo; namely, that competing demands affect erythroid and granulocytic differentiation (*1, 2*). Our results, however, are not compatible with a "three-tier" stem cell model (*3*) in which inducers act only on unipotent stem cells, unless these committed stem cells can again become pluripotent, in which case "commitment" has an altered meaning. Our findings do fit an alternative model in which pluripotent stem cells, once they start cycling, become sensitive to inducers through the transient appearance of specific receptors for the inducer during the cell cycle (*4*). In addition, these data confirm our previous results suggesting that erythropoietin can act at the pluripotent stem cell level by predisposing spleen colony-forming cells to erythroid colony formation at the expense of nonerythroid spleen colonies (*12*).

An alternative explanation of these results suggests that the committed, unipotent cells have receptors with specificities that overlap, so that the erythropoietin receptor can bind CSF and the CSF receptor can bind erythropoietin. The binding of the "wrong" inducer will not result in the initiation of the developmental process, but will prevent the binding of the "right" inducer. These two different models could, in principle, be distinguished by study of the binding of labeled inducer by means of autoradiography.

GARY VAN ZANT
EUGENE GOLDWASSER
Franklin McLean Memorial Research Institute and *Department of Biochemistry, University of Chicago, Chicago, Illinois 60637*

References and Notes

1. T. R. Bradley, W. Robinson, D. Metcalf, *Nature (London)* **214**, 511 (1967); P. F. Harris, R. S. Harris, J. H. Kugler, *Br. J. Haematol.* **12**, 419 (1966); S. Hellman and H. E. Grate, *Nature (London)* **216**, 65 (1967).
2. B. Kubanek, O. Bock, W. Heit, E. Bock, E. B. Harris, *Ciba Found. Symp.* **13**, 243 (1973); P. J. Smith and H. Ekert, *Lancet* **1976-I**, 776 (1976); H. N. Steinberg, E. S. Handler, E. E. Handler, *Blood* **47**, 1041 (1976); S. Hellman and H. E. Grate, *J. Exp. Med.* **127**, 605 (1968).
3. L. G. Lajtha, *Br. J. Haematol.* **29**, 529 (1975); ——— and R. Schofield, *Differentiation* **2**, 313 (1974); E. A. McCulloch, T. W. Mak, G. B. Price, J. E. Till, *Biochim. Biophys. Acta* **355**, 260 (1974); D. W. Golde and M. J. Cline, *N. Engl. J. Med.* **291**, 1388 (1974).
4. E. Goldwasser, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 2285 (1975).
5. A. L. Kretchmar, *Science* **152**, 367 (1966).
6. E. Goldwasser, J. F. Eliason, D. Sikkema, *Endocrinol.* **97**, 315 (1975); D. Metcalf, *J. Cell. Physiol.* **76**, 89 (1970).
7. E. R. Stanley and D. Metcalf, *Aust. J. Exp. Biol. Med. Sci.* **47**, 467 (1969).
8. G. Hodson and I. Eskuche, *Proc. Soc. Exp. Biol. Med.* **127**, 328 (1968); M. Gross and E. Goldwasser, *J. Biol. Chem.* **246**, 2480 (1971); Y. Matoth and E. Ben-Porath, *J. Lab. Clin. Med.* **54**, 722 (1959).
9. E. R. Stanley and P. M. Heard, *J. Biol. Chem.* **252**, 4305 (1977).
10. E. Goldwasser and C. K.-H. Kung, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 697 (1971).
11. B. S. Mitchell and J. W. Adamson, in *Erythropoiesis*, K. Nakao, J. W. Fisher, F. Takaku, Eds. (Univ. of Tokyo Press, Tokyo, 1975), p. 151.
12. G. Van Zant and E. Goldwasser, *J. Cell. Physiol.* **90**, 241 (1977).
13. We thank R. Stanley of the Albert Einstein College of Medicine, New York, for the partially purified CSF preparations, and N. Pech for excellent technical assistance. The Franklin McLean Memorial Research Institute is operated by the University of Chicago for Energy Research and Development Agency under contract EY-76-C-02-0069. This research was supported in part by grants CA-18375 and CA-14599 from the National Cancer Institute. G.V.Z. received NIH postdoctoral research fellowship 5 F22 AM 00421 and training grant HD-00298.

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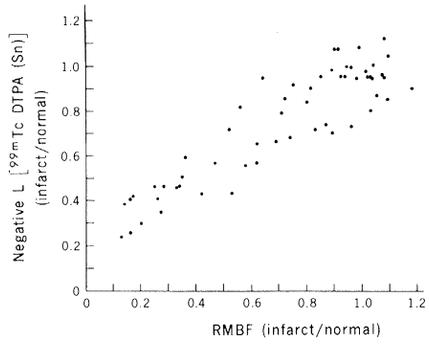
Liposome Accumulation in Regions of Experimental Myocardial Infarction

Abstract. *The uptake of liposomes bearing positive, negative, or no net charge on their membrane and containing a radioactive tracer, [^{99m}Tc]diethylenetriamine pentaacetic acid, was studied in 12 intact dogs 24 hours after the induction of myocardial infarction, and compared to the relative regional myocardial blood flow determined from radioactive microspheres. Positively charged and neutral liposomes concentrate in infarcted regions against a flow gradient, while negative liposomes are passively distributed according to regional blood flow. Because positively charged and neutral liposomes concentrate in infarct areas and have the ability to incorporate pharmacologic agents in their aqueous or lipid phase, they may serve as vehicles for drug delivery to infarct zones of low flow.*

Liposomes have been proposed as vehicles for delivery of therapeutic agents into cells. Other investigators have discussed the capacity of these lipid spheres to provide an enhanced mode of intracellular entry for metabolically and pharmacologically active substances such as enzymes, antineoplastic drugs, chelating agents, and hormones (*1*). In most studies, cell suspensions in vitro or tissue cultures were used, and attention was focused on mechanisms of liposome-cell interactions and characterization of elicited intracellular responses. Other workers have investigated the characteristics of the distribution of liposomes in normal and abnormal tissues in vivo. Recently, liposomes were found to be concentrated preferentially in certain tumors, a situation with major therapeutic implications. In fact, the administration of liposomes containing actinomycin D to tumor-bearing mice prolonged their survival compared to mice treated with the free chemotherapeutic agent alone (*2*).

Acute myocardial infarction secondary to coronary artery occlusion is a condition that might benefit from the enhanced delivery of pharmacologic agents. The work of Maroko and Braunwald (*3*) suggests that the degree of ischemic injury and subsequent necrosis resulting from myocardial infarction is not constant, but rather is subject to therapeutic modification. The selective concentration of appropriate therapeutic agents, particularly in infarct regions of markedly reduced regional blood flow,



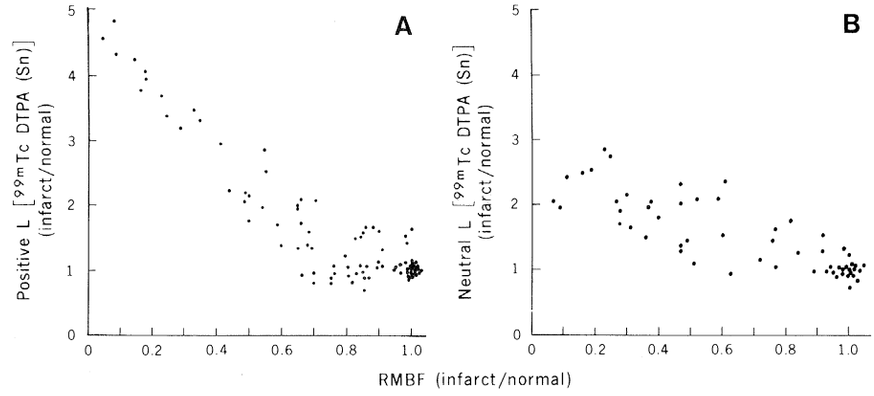


Fig. 1 (left). Relation between the uptake of negatively charged liposomes (L) [^{99m}Tc DTPA (Sn)] and the distribution of ^{85}Sr-labeled microspheres in the left ventricle myocardium of dogs subjected to experimental infarction. Activities are expressed as ratios between infarct sample and the mean of five normal samples in each study. The infarct to normal ratio obtained from the radioactivity of the ^{85}Sr-labeled microspheres is an estimation of relative regional myocardial blood flow (*RMBF*). A positive correlation is shown between the liposome concentration and the relative regional myocardial blood flow ($N = 58, r = 0.82, y = 0.679 \times +0.25$). Fig 2 (right). Relation between the uptake of (A) positively charged and (B) neutral liposomes [^{99m}Tc DTPA (Sn)] and the distribution of ^{85}Sr-labeled microspheres in myocardium. (A) When the relative regional myocardial blood flow decreases, the concentration of positively charged liposomes increases ($N = 94, r = -0.88, y = -2.8 \times +3.8$). (B) A similar increase is observed with neutral liposomes ($N = 54, r = -0.80, y = -1.37 \times +2.42$).

might provide the optimum circumstance for salvaging potentially retrievable ischemic myocardium. We now report that intravenously administered liposomes, potential carriers of pharmacologically and metabolically active agents, distribute within the canine left ventricle subjected to acute infarction in a manner resulting in preferential accumulation within the infarct zone. This phenomenon is also related to the net surface charge of the liposomes such that liposomes with a net positive charge or with no net charge on their lipid membrane concentrate in infarct regions, while liposomes with a net negative charge do not.

Liposomes were prepared according to standardized techniques that have established that the addition of an anionic phospholipid (dicetylphosphate) or a cationic phospholipid (stearylamine) confer either negative or positive net surface charge to the liposome membrane, respectively (*4*).

Neutral liposomes were prepared with lecithin and cholesterol (molar percentages 84 : 16); negatively charged liposomes with lecithin, cholesterol, and diacetylphosphate (molar percentages 70 : 10 : 20); and positively charged liposomes with lecithin, cholesterol, and stearylamine (80 : 10 : 10) (*5*). After rotatory evaporation of the lipid mixture in chloroform, the dry lipids were resuspended in a saline solution of [λ^{99m}Tc]λ diethylenetriamine pentaacetic acid (DTPA) and sonicated for 5 minutes in a bath sonicator at room temperature (*6*). This was followed by filtration of the lipids through a 1.2-μm Millipore filter. The nonentrapped radioactive tracer was removed by column chromatography in Sepharose 6B (positive and negative liposomes) or Sephadex G-200 (neutral liposomes) or by centrifugation at 1300g for 15 minutes (neutral liposomes). In the last case, the supernatant was removed and the liposome pellet was resuspended in saline and centrifuged again. The procedure was repeated two times, and before use, the liposomes were forced through a 1.2-μm Millipore filter (*7*). Radioactively labeled liposomes of approximately 1 μm in diameter, with either net positive, negative, or no net surface charge were administered intravenously to 12 dogs (*8*), 24 hours after the induction of embolic closed-chest anterior wall myocardial infarction created as described (*9*). Two hours after the injection of liposomes, 2×10^6 to 4×10^6 ^{85}Sr-labeled carbonized microspheres (15 ± 3 μm in diameter) (*10*) were injected into the left atrium for measurement of relative regional myocardial blood flow (*11*). Five minutes after microsphere injection, the animals were killed.

The radioactivity from the liposomes and the ^{85}Sr-labeled microspheres was measured in both infarcted and normal left ventricle regions (*12*). Infarct to normal ratios (I/N) for transmural segments as well as endocardial and epicardial portions were calculated for both liposomes and microspheres. The regional myocardial concentration of liposomes could then be directly related to corresponding regional myocardial blood flow in the same sample.

Liposomes with negative surface charge did not become concentrated in regions of ischemia or infarction. In fact, myocardial uptake of negative liposomes correlated positively with microsphere estimates of relative regional myocardial blood flow (Fig. 1). In contrast to negative liposomes, both positive and neutral liposomes became concentrated in areas of ischemia and infarction. This accumulation was routinely noted in regions with reduced regional myocardial blood flow, and as regional myocardial blood flow decreased, liposome concentration increased (Fig. 2). The transmural distribution of liposomes within the infarct zone further demonstrated this accumulation against a flow gradient (Fig. 3). Within the infarct zone, as expected, the regional myocardial blood flow, as determined from the labeled microspheres, demonstrated relative subendocardial ischemia with endocardial to epicardial (endo : epi) flow ratios consistently less than one. However, the endo : epi ratio for positive liposomes (mean $\pm$ standard error) was 1.27 ± 0.05, whereas the corresponding endo : epi ratio for the microspheres in the same animals was 0.65 ± 0.09 ($P < .001$). For neutral liposomes the endo : epi ratio was 1.36 ± 0.06, with the corresponding endo : epi ratio for labeled microspheres being 0.27 ± 0.05 ($P < .001$). For negative liposomes, the endo : epi ratio was 0.75 ± 0.04, whereas the ^{85}Sr ratio was 0.53 ± 0.08 ($P < .05$) (two-tailed significance test). In the last case, the small but significant difference observed between the two endo : epi ratios is not an indication of accumulation of negative liposomes in areas of endocardial ischemia, but may reflect the disparity in diameter between liposomes (approximately 1 μm) and microspheres (15 ± 3 μm). The effect of size on the endo : epi distribution of microspheres has been documented (*13*).

These findings indicate that the concentration of liposomes in ischemic or in-

farcted tissue is related to the surface properties of the liposomes. Liposomes prepared with diacetylphosphate and bearing a net negative charge do not become concentrated in infarcted areas and are distributed passively in response to regional myocardial blood flow, whereas positively charged and neutral liposomes show a preferential myocardial uptake in regions with diminished regional myocardial blood flow. These data corroborate the demonstration in vitro that the composition of the liposomic membrane influences liposome-cell interactions (*14*). Studies in vivo also have shown surface charge–dependent differences in organ distribution and rate of removal of liposomes from the circulation (*15, 16*).

It can be argued that by detecting the activity of ^{99m}Tc DTPA we do not follow the fate of the liposomic membrane; since even though the free tracer was removed by chromatography or centrifugation, the labeled compound contained in the aqueous phase of the liposomes and adsorbed on the surface of liposomes may have been released in the bloodstream after administration. However, it has been demonstrated that (i) liposomes carry their content in the bloodstream without significant interaction with the environment (*17*); (ii) liposomes successfully deliver their content into the cytoplasm after endocytosis or fusion with cellular membranes (*18*); and (iii) positive, negative, and neutral liposomes labeled with ^{99m}Tc DTPA dramatically change the organ distribution of ^{99m}Tc DTPA in normal mice and dogs (*16, 19*). It is therefore reasonable to assume that in our experiments the distribution of ^{99m}Tc radioactivity represents the distribution of liposomes.

The type of cells responsible for liposomal uptake in ischemic or infarcted myocardium, the degree of cellular damage needed to induce such uptake, and the mechanisms for the selective concentration of liposomes with varying surface charges are all unknown. It is conceivable that the regional uptake may be a reflection of the prolonged residence time of liposomes in regions of low blood flow. Some additional mechanisms that should be considered include (i) variations in capillary permeability owing to direct alteration of endothelial cells or to changes in the ionic composition of surrounding tissues; (ii) phagocytosis of liposomes by leukocytes followed by leukocyte-liposome migration into infarcted regions; (iii) preferential regional destruction owing to lysosomic enzymes followed by release of intraliposomic content; and (iv) direct penetration of liposomes into the cytoplasm of ischemic

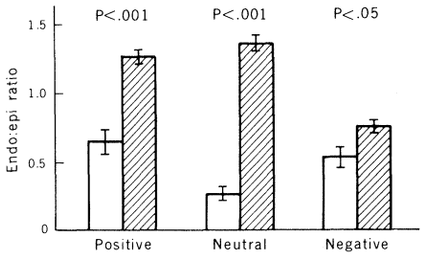


Fig. 3. Endocardium to epicardium ratios within the infarct zone for positive, neutral, and negatively charged liposomes (hatched bars) and for ^{85}Sr-labeled microspheres (open bars). Positive and neutral liposomes accumulate in endocardium (endo : epi ratio > 1), while the microsphere regional myocardial blood flow demonstrates a relative subendocardial ischemia (endo : epi ratio < 1). The small but significant difference between the transmural distribution of negatively charged liposomes and corresponding microspheres is explained by the disparity in diameter of liposomes (~1 μm) and microspheres (15 ± 3 μm) (see text). In contrast, in normal myocardium, endo : epi ratios for positive, negative, and neutral liposomes were 1.09 ± 0.04, 0.77 ± 0.02, and 1.13 ± 0.04, respectively.

or infarcted cells, facilitated by structural alteration of the cell membrane, thereby exposing strongly negative intracytoplasmic proteins to the extracellular compartment (*20*).

Irrespective of the mechanisms of uptake, the radioactive tracer contained in positive and neutral liposomes can reach areas of myocardial ischemia and concentrate therein. In these experiments, liposomes delivered their content to the affected myocardial regions, thereby demonstrating their potential use as vehicles for drug delivery. The magnitude of entrapment of water-soluble drugs in liposomes is limited by the volume of the aqueous compartment of the liposomes. Although the aqueous phase volume may be increased, the size of that compartment will always present a limit to the amount of water-soluble drug that can be introduced. In our experiments the entrapment of ^{99m}Tc DTPA was never beyond 3 percent of the activity initially added. This limitation does not exist when lipid-soluble substances are used because they can be readily incorporated into the lipid phase of the liposome. In this last case incorporation will approach 100 percent. The effect that lipid-soluble compounds incorporated in the lipid membrane may have on the stability of liposomes should be considered for each particular case.

It is conceivable that, as carriers of antiarrhythmic drugs, liposomes could ensure preferential drug accumulation in severely ischemic regions which are the anatomic site of origin of potentially lethal ventricular arrhythmias. The capacity of liposomes to reach these myocardial regions also might be utilized to deliver other compounds that could lead to increased regional myocardial viability following infarction. Several pharmacologic agents have been suggested for this purpose. The administration of hyaluronidase and glucose-insulin-potassium has been shown to reduce the size of experimental myocardial infarction (*21*). Similarly, the use of corticosteroids has been reported to modify the extent of necrosis, most probably through the stabilization of lysosomal and cellular membranes (*22*). Finally, enhanced local delivery of metabolically active agents such as high-energy phosphates, β-adrenergic blocking agents, or intracellular buffers to offset regional acidosis might also be considered.

VICENTE J. CARIDE
Department of Radiology, Yale University School of Medicine, New Haven, Connecticut 06504
BARRY L. ZARET
Departments of Medicine and *Radiology, Yale University School of Medicine*

References and Notes

1. G. Sessa and G. Weissman, *J. Biol. Chem.* **245**, 3295 (1970); D. Papahadjopoulos, G. Poste, W. J. Vail, J. L. Biedler, *Cancer Res.* **36**, 2988 (1976); Y. E. Rahman and B. J. Wright, *J. Cell Biol.* **65**, 112 (1975); G. Gregoriadis, *N. Engl. J. Med.* **295**, 704 (1976); *ibid.*, p. 765.
2. G. Gregoriadis, E. J. Wills, C. P. Swain, A. S. Tavill, *Lancet* **1974-I**, 1313 (1974); G. Gregoriadis, *FEBS Lett.* **36**, 292 (1973); *Biochem. Soc. Trans.* **2**, 117 (1974); _____ and D. E. Neerunjun, *Res. Commun. Chem. Pathol. Pharmacol.* **10**, 351 (1975).
3. P. R. Maroko and E. Braunwald, *Ann. Intern. Med.* **79**, 720 (1973).
4. G. Sessa and G. Weissman, *J. Biol. Chem.* **245**, 3295 (1970); A. D. Bangham, *Adv. Lipid Res.* **1**, 65 (1963).
5. The lecithin (egg) was obtained from Gibco; the stearylamine (*N*-octadecylamine) from ICN K&K Laboratories; the dicetylphosphate from Sigma; and the cholesterol from Fisher Scientific.
6. The ^{99m}Tc DTPA was obtained as a kit from Diagnostic Isotopes, and contained 5 mg of DTPA and 0.25 mg of stannous chloride. A Branson bath sonicator was used, at 50 to 55 khz.
7. In these experiments, the percentage of the added ^{99m}Tc DTPA (Sn) entrapped into liposomes averaged 1.8 ± 0.6 for positive liposomes, 0.5 ± 0.1 for neutral liposomes, and 2 ± 0.6 for negative liposomes (mean ± standard error).
8. Twelve dogs were used for this study: five were injected with positive liposomes, four with neutral liposomes, and three with negative liposomes. Of the four dogs injected with neutral liposomes, two were injected with liposomes in which the free tracer was removed by centrifugation.
9. B. L. Zaret, V. C. DiCola, R. K. Donabedian, S. Puri, S. Wolfson, G. S. Freedman, L. S. Cohen, *Circulation* **53**, 422 (1976).
10. Carbonized microspheres, labeled with ^{85}Sr (3M Corporation).
11. R. J. Domenech, J. I. E. Hoffman, M. I. Noble, K. B. Sauders, J. R. Henson, S. Subijanto, *Circ. Res.* **25**, 581 (1969); L. C. Becker, N. J. Fortuin, B. Pitt, *ibid.* **28**, 263 (1971).
12. After the dog was killed, the heart was removed and the left ventricle sectioned in approximately 20 samples. Each sample was divided into epicardium and endocardium (0.5 to 1 g, sample weight) and placed in a scintillation vial. All samples were counted in a three-channel spectrometer. Appropriate corrections for ^{85}Sr contribution at the ^{99m}Tc setting and for the physical decay of ^{99m}Tc were made. Infarct samples were from the visually apparent central infarct zone.

Normal tissue was obtained from the posterolateral wall (uninvolved circumflex coronary artery distribution). No attempt was made to define a border "ischemic" zone. Evidence of myocardial infarction and necrosis was confirmed histopathologically [V. C. DiCola, S. E. Downing, R. K. Donabedian, B. L. Zaret, *Cardiovasc. Res.* **11**, 141 (1977)].
13. N. J. Fortuin, S. Kaihara, L. C. Becker, B. Pitt, *ibid.* **5**, 331 (1971).
14. D. Papahadjopoulos, G. Poste, B. T. Schaeffner, W. J. Vail, *Biophys. Biochim. Acta* **352**, 10 (1974); F. J. Martin and R. C. McDonald, *J. Cell Biol.* **70**, 494 (1976); *ibid.*, p. 506; *ibid.*, p. 515; G. Poste and D. Papahadjopoulos, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1603 (1976).
15. R. L. Juliano and D. Stamp, *Biochem. Biophys. Res. Commun.* **63**, 651 (1975); M. M. Jonah, E. A. Cerny, Y. E. Rahman, *Biophys. Biochim. Acta* **401**, 336 (1975).
16. V. J. Caride, paper presented at the Radiological Society of North America meeting, Chicago (1976).
17. I. R. McDougall, J. K. Dunnick, M. G. McNammee, J. P. Kriss, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3487 (1974); G. Gregoriadis and A. C. Allison, *FEBS Lett.* **45**, 71 (1974).
18. G. Gregoriadis and B. E. Ryman, *Biochem. J.* **129**, 123 (1972); G. Weissman, D. Bloomgarden, R. Kaplan, C. Cohen, S. Hoffstein, T. Collins, A. Gottleib, D. Nagle, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 88 (1975).
19. V. J. Caride, W. Taylor, J. A. Cramer, A. Gottschalk, *J. Nucl. Med.* **17**, 1067 (1976).
20. B. F. Trump and W. J. Mergner, in *The Inflammatory Process*, B. W. Zweifach, L. Grant, R. T. McCloskey, Eds. (Academic Press, New York, ed. 2, 1974), chap. 3.
21. P. R. Maroko, P. Libby, B. E. Sobel, C. M. Bloor, H. D. Sybers, W. E. Shell, J. W. Covell, E. Braunwald, *Circulation* **45**, 1160 (1972); L. H. Opie, K. Bruyneel, P. Owen, *ibid.* **52**, 49 (1975).
22. P. Libby, P. R. Maroko, C. M. Bloor, B. E. Sobel, E. Braunwald, *J. Clin. Invest.* **52**, 599 (1973); J. A. Spath, D. L. Lane, A. M. Lefer, *Circ. Res.* **35**, 44 (1974); G. Weissman, *Circulation* **53**, I-171 (1976).
23. Supported by American Heart Association grant 75-523; National Heart, Lung, and Blood Institute contract 1-HV52988; the Connecticut Heart Association; and Heart Association of Greater Hartford grant 11-204-767. B.L.Z. is an established investigator of the American Heart Association. We thank M. Addabo and J. Grella for technical assistance.

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Screening for Twin Pregnancy

Abstract. *A group of 590 women who, 4 to 5 weeks after their last menstrual period, were confirmed to be pregnant, as measured by the human chorionic gonadotropin (hCG) by radioreceptor assay. Nine of these women had serum hCG levels approximately twofold higher than the others and were suspected of having twin pregnancy. When these women were tested at 12 weeks of gestation, pelvic sonography confirmed twin pregnancies in all the nine cases. Serum hCG levels thus provide a simple, rapid, and easy method to detect twin pregnancy.*

Fetal risk in twin pregnancies are three to four times higher than in single gestation (*1*). The combined effects of prematurity and intrauterine retardation are the main causes of perinatal wastage in multiple births. Moreover, maternal complications in twin pregnancy are increased and include preeclampsia, polyhydramnios, anemia, and intrapartum complications (*2*). The prognosis for twin pregnancy can be considerably improved by early diagnosis and adoption of prophylactic measures. For example, in one study, the incidence of premature labor was reduced to 2 percent in patients with strict bed rest, as compared to 12 percent in the other patients with twins (*3*). At present, 50 percent of all twin deliveries are discovered only at delivery (*4*). Serum titers of human placental lactogen have been shown to be diagnostic of twins at 29 weeks of gestation (*5*). Pelvic ultrasound can detect twins at 12 weeks of gestation (*1*); however, sonography is expensive, time-consuming, and not widely available. An earlier screening test, therefore, is needed to provide for optimal care in twin pregnancy.

Seven days after conception, human chorionic gonadotropin (hCG) can be detected in serums of pregnant women by radioimmunoassay and radioreceptor-assay (RRA), which detects hCG or luteinizing hormone (or both) (*6*). During the last 3 years, more than 5000 women were screened for pregnancy with the RRA for hCG. There has been nearly 100 percent reliability in the detection of pregnancy at the time of missed period. The level of hCG has been a good index for the size, weight, and function of the placenta in pregnancy (*7, 8*).

From July to November 1976, a total of 590 serum samples obtained from women 4 to 5 weeks after their last menstrual period were positive for hCG by the RRA. The hCG levels of nine of these samples were significantly higher than those of the other 581 samples. At least four subsequent determinations were obtained on each of these nine patients during the first 70 days of gestation. Pelvic sonography was performed in all nine of these patients. Fifteen of the 581 women, with normal hCG titers for 4 to 5 weeks of gestation, were followed with weekly determinations of serum hCG. All 15 delivered single infants at full term. Therefore, their serial hCG levels were used as the control.

As is shown in Table 1, the levels of hCG at the time of the missed period (33 days from the last menstrual period) were, approximately, twofold greater in the women carrying twins than in those carrying single infants. In the cases of single-infant gestation, hCG rose from 1 to 1000 ng/ml within 30 days, whereas in the women with twin gestations hCG rose from 1 ng/ml to 1000 ng/ml, within 10 days [1 ng of hCG = 12 milli-international units (mIU)]. The hCG reached a plateau at 10 weeks in the women with single gestations with a range of 8,000 to 12,000 ng/ml. The hCG plateau in the women carrying twins also occurred at 10 weeks but with a range of 20,000 to 40,000 ng/ml. All of the nine women who showed the higher hCG levels were confirmed by pelvic sonography to have twins. These observations show that the determination of serum hCG early in pregnancy could be used as a screening test for twins and would be useful in patients who have a high probability of carrying twins, such as in cases of ovulation induction. Therefore, the physician is able to take prophylactic measures at the earliest possible time.

Table 1. Comparison of hCG (1 ng hCG = 12 milli-international units) levels in single and twin pregnancies. Abbreviations: LMP, last menstrual period; LH, luteinizing hormone.

Days from LMP	Single gestation hCG or LH (ng/ml)		Twin gestations hCG or LH (ng/ml)	
	Mean*	Range	Mean†	Range
28	5.39	0.78 to 10	5.39	0.79 to 10
33	37.5	25 to 50	125	100 to 150
36	125	100 to 150	1,600	200 to 300
40	300	200 to 400	4,862	725 to 9,000
45	3,000	1,000 to 5,000	10,500	6,000 to 15,000
70	10,000	8,000 to 12,000	34,500	29,000 to 40,000

*Mean from 15 women with normal single gestation. †Mean from nine women with twin gestation.

LOIS JOVANOVIC
ROBERT LANDESMAN, BRIJ B. SAXENA
Cornell University Medical College, New York 10021

References and Notes

1. "International twin study," *Acta Geet. Med. Gemell* **23**, 53 (1974).
2. W. Powers, *Obstet. Gynecol.* **42**, 795 (1973).
3. *Lancet*, **1975-I**, 27 (1975).
4. K. Ahlstrom, *Lakartidningen* **71**, 3089 (1974).
5. M. Magiste and H. Von Schenck, *Am. J. Obstet. Gynecol.* **126**, 697 (1976).
6. B. B. Saxena, H. Hason, F. Haour *Science* **184**, 793 (1974); T. Kosasa *et al. Am. J. Obstet. Gynecol.* **119**, 784 (1974).
7. T. P. Rosal, B. B. Saxena, R. Landesman, *Fertil. Steril.* **26**, 1105 (1975).
8. R. Landesman and B. B. Saxena, *ibid.* **27**, 357 (1976).

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