Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Enzymes Immobilized by Microencapsulation Within Spherical Ultrathin Polymeric Membranes

T. M. S. Chang[a]
[a] Faculty of Medicine McGill University Montreal, Quebec, Canada

To cite this Article Chang, T. M. S.(1976) 'Enzymes Immobilized by Microencapsulation Within Spherical Ultrathin Polymeric Membranes', Journal of Macromolecular Science, Part A, 10: 1, 245 — 258
To link to this Article: DOI: 10.1080/00222337608068098
URL: http://dx.doi.org/10.1080/00222337608068098

J. MACROMOL. SCI.--CHEM., A10(1&2), pp. 245-258 (1976)

ENZYMES IMMOBILIZED BY MICROENCAPSULATION WITHIN SPHERICAL ULTRATHIN POLYMERIC MEMBRANES

T. M. S. Chang

Faculty of Medicine
McGill University
Montreal, Quebec, Canada

INTRODUCTION

Biological cells in nature have diameters of about 7 to 50 μm, with a cell membrane of about 0.01 μm in thickness. This means that the total membrane area available for diffusion can be extremely large in comparison to the volume and that the cell membrane can also be very thin without the loss of mechanical strength. In addition, rapid mixing is possible within the individual microscopic compartments. Biological cells have very complex and selective enzyme systems to act on substrates crossing the cell membrane. Artificial cells in the form of spherical ultrathin polymeric membrane envelopes containing enzymes have been prepared in this laboratory with some of these properties [1-3]. Detailed papers and upadated information published since then on this subject have been described in a recent monograph [4].

PREPARATION

The cell model system first prepared in this laboratory in 1956 consisted of red blood cell hemolysate enclosed within spherical ultrathin collodion membranes of cellular dimensions. Each "artificial cell" is of cellular dimension containing all the protein and enzyme systems of red blood cells, except that the red blood cell membrane has been replaced with an ultrathin collodion membrane. In

this initial preparative procedure there are three main steps: (I) The aqueous protein and enzyme solution is emulsified in a water-immiscible solution to form aqueous microdroplets. (II) With the addition of a polymer solution to the stirred emulsion, a membrane is formed on the surface of each microdroplet and then allowed to set in an appropriate solution. (III) Finally, the microencapsulated red blood cell hemolysate formed is dispersed in an aqueous phase. With modifications, this general procedure has been varied for the preparation of artificial cells with a great variety of membrane material, content, size, and configuration. The preparative procedures have been updated and described in detail [4].

Briefly, the first approaches [1,2,4] involved organic phase separation. Polymers dissolved in a water-immiscible fluid are deposited around each aqueous microdroplet dispersed in the water-immiscible fluid. This is one of the most convenient ways of preparing microencapsulated enzymes. The details have been updated and simplified [4]. The best approach is to use the 10 g % hemoglobin solution for microencapsulation, and any enzymes to be enclosed could be added to the hemoglobin solution, since without the 10 g % hemoglobin solution, which is a basic component of the cell model, a great deal of variation and modification of the preparative procedure would be needed. Enzymes, proteins, or other material for microencapsulation are added to the 10 g % hemoglobin solution before carrying out the microencapsulation procedure.

Another approach [2-4] is to use interfacial polymerization. A diamine dissolved in the aqueous phase of the 10 g % hemoglobin reacts at the interphase of each aqueous microdroplet with sebacoyl chloride in the organic phase to form a membrane. Nylon membrane is used most commonly for this, but other membranes could also be formed, for instance, polyamides, polyureas, polyurethanes, polysulfonamides, and polyphenylesters [4]. Again, in this typical example, it is most convenient to use a 10 g % hemoglobin solution and to add to this the enzymes or other proteins that one wants to immobilize within the microcapsules. This chemical procedure, un-

like the first procedure of organic phase separation, may inactivate some enzyme systems.

A third approach [4,5] would be by secondary emulsion. Here a fine emulsion of enzyme solution is dispersed in an organic phase containing a polymer. The organic phase containing the fine aqueous microdroplets is then dispersed in the form of large droplets in an aqueous phase. The organic phase containing the polymer solutions is then solidified to form solid microspheres, each containing a number of aqueous microdroplets. Unlike the two examples described earlier, this procedure results in solid polymer microspheres, each of which contains several aqueous microdroplets. A typical example is the use of silicone rubber to microencapsulate red blood cell hemolysate [5]. Other polymers include the use of cellulose membrane and other membranes [4,5]. Another procedure [7] is the emulsification of enzyme solution in a polymer organic solution, but instead of forming microspheres the polymer solution containing the enzyme microdroplets is extruded in the form of fibers. Further extensions include the microencapsulation of enzymes within liposomes [8]. However, in this case the enzyme is entrapped between onion-skin-like multiple laminar lipid. Hemolysate has also been microencapsulated within spherical ultrathin lipid membrane [9]. Enzymes have also been microencapsulated within liquid membranes [10].

Since the "artificial cell" is a general concept [4], the artificial cells prepared by the procedures that have been described are only some examples for demonstrating this concept. It is expected that modifications or extensions of these procedures can be made available to further demonstrate or improve the preparation of different types of artificial cells.

PROPERTIES

The ultrathin membranes (0.02 μm) and the large surface to volume relationship of artificial cells (2 m^2 area in 10 ml of 20 μm diameter artificial cells) results in extremely high transport rates across the artificial cell membranes. Thus 10 ml of these 10 μm

diameter spherical ultrathin polymer membranes has a theoretical transport capacity 200 times higher than that of a whole standard 1 m^2 dialysis machine [5,11]. The enclosing polymeric membrane of artificial cells is prepared in such a way that while impereable to macromolecules or suspensions, it is extremely permeable to most of the solutes normally present in the biological fluid. Enveloped by the spherical ultrathin polymer membrane is either an aqueous solution or a suspension of biologically active material like enzyme systems. While remaining at all times enveloped by the enclosing polymeric membrane and prevented from coming into direct content with the external environment, the enclosed enzyme systems act on external permeant substrates diffusing into the artificial cells. This means that enzymes cannot leak out of the spherical ultrathin polymeric membrane envelopes or come in contact with external macromolecules like antibodies. However, permeant substrates can equilibrate rapidly across the enclosing membranes to be acted on by the enclosed enzymes, and the products can diffuse out of the microcapsules. The permeability characteristics of the polymeric membranes can be varied at will as to porosity, charge, composition, and lipid contents. In addition, the polymeric membranes can also be made biologically compatible [4].

MICROENCAPSULATED ENZYMES

Table 1 lists enzymes and proteins which have been immobilized by microencapsulation. Additional pertinent references are also to be noted [16,18,32,36-38,41,49-51,53-55]. Thus hemoglobin, albumin, uricase, urease, catalase, asparaginase, carbonic anhydrase, trypsin, lactase, and many other enzymes have been immobilized by microencapsulation. Even complex enzyme systems like the contents of red blood cells and muscle cells have been microencapsulated (Figs. 1-3). The microencapsulated enzymes do not leak out but act efficiently on permeant substrates. Products formed can leave the microcapsules. Most enzyme activities are retained after microencapsulation. The kinetics of reaction are governed by a large number of factors: concentration of enzymes

TABLE 1

Enzymes and Proteins Immobilized within Semipermeable Microcapsules

	References
Enzymes and hemoglobin of red blood cell hemolysate	1-3, 6, 9, 12, 13, 28
Urease	1, 3, 5, 6, 10, 14, 15, 17, 28-31, 34, 35, 44
Carbonic anhydrase	2, 19
Uricase	2, 3
Trypsin	2
Catalase	6, 20, 42, 45, 46
Asparaginase	4, 11, 21, 22, 30, 33-35 39, 43, 47, 48, 52
Albumin	2, 3, 23
Lipase	6
Zymase complex (yeast)	24
Muscle extract	24
Phenolase	25
Nitrate reductase	26
β-Fructofuranosidase	8
β-Galactosidase	27, 39, 40, 52

in the microcapsules, permeability of membrane to the substrates and products, charge of membrane, composition of membranes, and other factors.

Enzymes can be stabilized by microencapsulation with a high concentration of protein in the form of the 10 g % hemoglobin solution [3,30,48]. Proteins cannot leak out of semipermeable microcapsules even when the microcapsules themselves are dispersed in a very dilute suspension. Thus, unlike native enzymes in a dilute solution, the microencapsulated enzyme is at all times retained in an intracellular environment with a high concentration of protein. In this way the stability of the enzyme is much greater than the enzyme in the dilute solution. For example, the half-life of

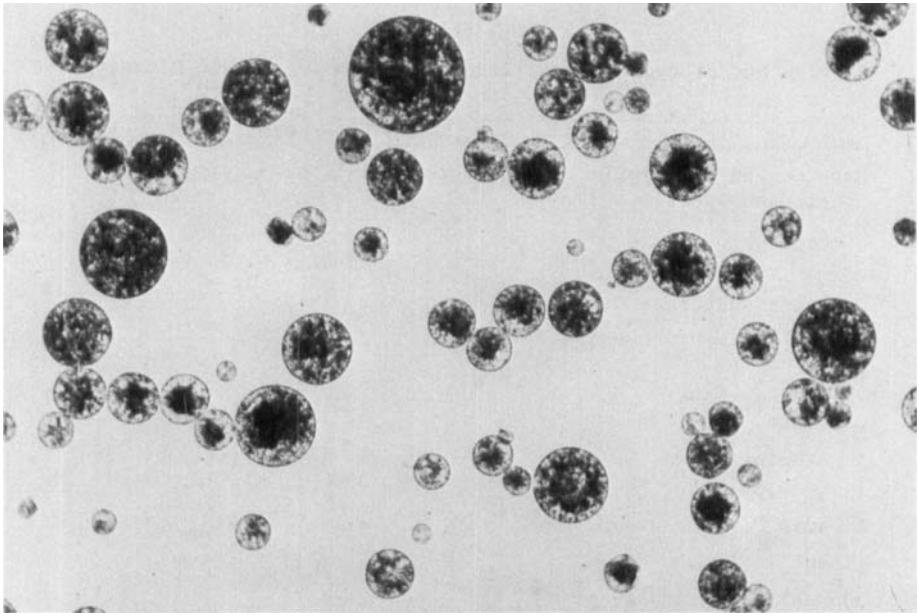

Fig. 1. Enzyme immobilized by microencapsulation within spherical ultrathin polymeric membranes. Mean diamter: 100 μm. spherical cellulose nitrate membrane of 0.02 μm thickness enveloping a complex enzyme system.

catalase in free solution is 15 days when stored at 4°C. When microencapsulated with a high concentration of protein and stored at 4°C, it retained more than 90% of its initial activity for more than 100 days. The half-life of asparaginase in free solution was 20 days when stored at 4°C, but when microencapsulated with a high concentration of protein the enzyme retained 100% of its initial activity for more than 100 days [4,39,48]. Urease and trypsin also become more stable after microencapsulation with a high concentration of protein. Enzymes can also be stabilized by directly microencapsulating insolubilized enzymes [4,34]. An easier way to insolubilize microencapsulated enzymes is to microencapsulate the enzyme at a very high concentration. Following this, it is treated

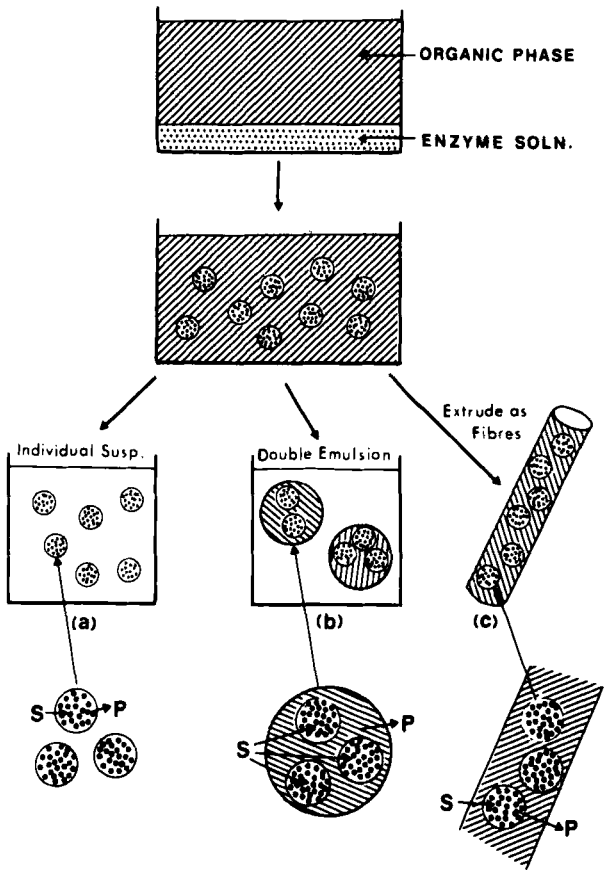

Fig. 2. Schematic representation of the preparation of spherical ultrathin polymeric membranes containing (a). (b) and (c) are modifications and extensions to form solid spheres (b) or fibers (c).

with glutaraldehyde, thereby cross-linking the enzymes within the microcapsule [48]. In this way, microencapsulated catalase and asparaginase retained more than 90% of their original activity after storage for more than 100 days at 4°C [48]. At body temperature of 37°C, the microencapsulated enzymes are also more stable.

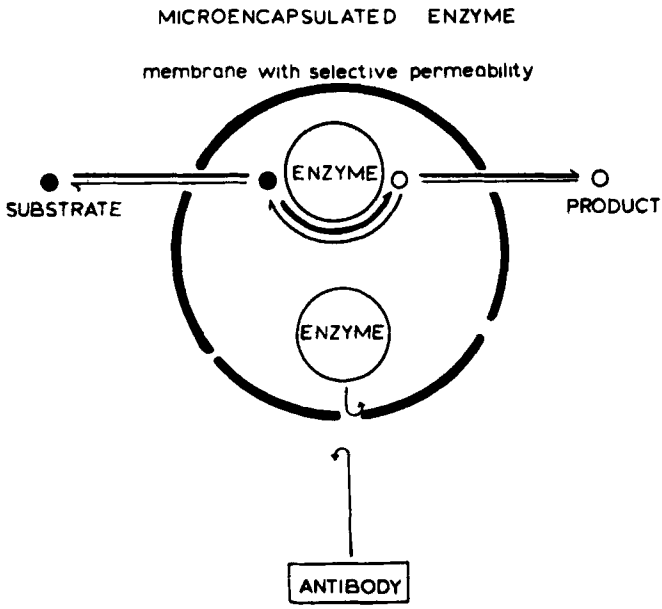

Fig. 3. Schematic representation of the action of microencapsulated enzymes. Enzyme does not leak out, but acts on permeant substrates. Antibody cannot enter to adversely affect the enzyme.

Microencapsulated enzymes have been injected intraperitoneally or used in extracorporeal shunts [4]. These microencapsulated enzymes did not leak out of the microcapsules but continued to act on substrates diffusing into the microcapsules. Urease acted on body urea [4]; microencapsulated catalase acted efficiently in replacing the deficient enzyme in an inborn error of metabolism; acatalasemia [4] and microencapsulated asparaginase acted efficiently in suppressing the growth of an asparagine-dependent lymphosarcoma [4]. Microencapsulated enzymes remain inside the artificial cells after injection. They are not inactivated by cells or enzymes in the body and are not removed rapidly. As a result, injected microencpasulated enzymes, such as catalase and asparaginase, acted significantly longer than when the same enzymes were injected in

TABLE 2

Stages of Enzyme Research

I.	Research on enzymes in dilute solution.
II.	Research on enzymes immobilized by covalent linkage, adsorption, or gel-entrapment as models of membrane-bound enzymes.
III.	Research on enzymes immobilized by microencapsulation within spherical ultrathin polymer membranes as models of intracellular enzymes (artificial cells).

the free form [4]. Furthermore, microencapsulated enzymes, unlike free enzymes, are prevented from taking part in immunological reactions [4,42].

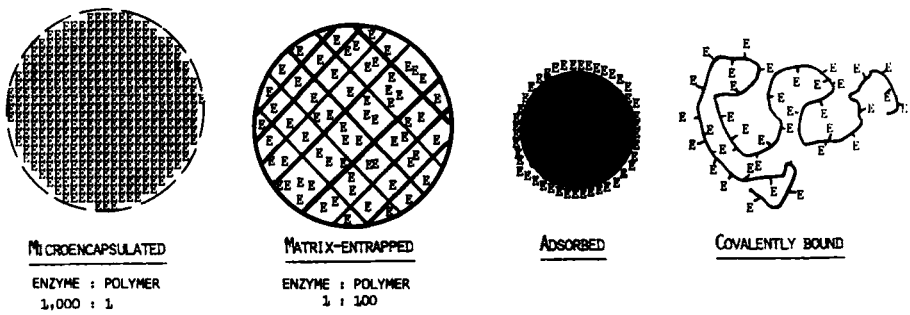

Fig. 4. Stages of enzyme research, illustrating the relative amounts of matrix support (■) and enzyme (E). Stage II: matrix entrapped, adsorbed, and covalently bound. Stage III: microencapsulated.

GENERAL

Enzyme research has proceeded from dilute enzyme solutions to the second level of gel-entrapped, adsorbed, and covalently bound enzymes as a model for membrane-bound enzymes, and finally, with the application of advances in polymer chemistry, we have demonstrated the feasibility of proceeding to the level of artificial cells containing enzymes as models of biological cells (Table 2, Fig. 4). With the experimental demonstration of the possible application of this spherical ultrathin polymeric system for basic research, applied research, and clinical use, further efforts are indicated, especially to apply the enormous amount of knowledge in polymer chemistry to this system.

ACKNOWLEDGMENT

This work was supported by the Medical Research Council of Canada (MRC-MT-2100).

REFERENCES

[1] T. M. S. Chang, "Hemoglobin Corpuscles," Report of research project for B.Sc. Honors Physiology, McGill University, Montreal (1957).

[2] T. M. S. Chang, "Semipermeable Microcapsules," Science, 146, 524 (1964).

[3] T. M. S. Chang, F. C. MacIntosh, and S. G. Mason, "Semipermeable Aqueous Microcapsules: Preparation and Properties," Can. J. Physiol. Pharmacol., 44, 115 (1966).

[4] T. M. S. Chang, Artificial Cells, monograph, Thomas, Springfield, Illinois, 1972, p. 207.

[5] T. M. S. Chang, "Semipermeable Aqueous Microcapsules ("Artificial Cells"): with Emphasis on Experiments in an Extracorporeal Shunt System," Trans. Am. Soc. Artif. Intern. Organs, 12, 13 (1966).

[6] M. Kitajima, S. Miyano, and A. Kondo, "Studies on Enzyme-Containing Microcapsules," J. Chem. Soc. Japan, Ind. Chem. Soc., 72, 493 (1969).

[7] W. Marconi, C. Saronio, M. Salmona, E. Mussini, and S. Garattini, Insolubilised Enzymes, Raven, New York, 1974.

[8] G. Gregoriadis and B. E. Ryman, "Fate of Protein-Containing Liposomes Injected into Rats. An Approach to the Treatment of Storate Diseases," Eur. J. Biochem., 24, 485 (1972).

[9] P. Mueller and D. O. Rudin, "Resting and Action Potentials in Experimental Bimolecular Lipid Membranes," J. Theor. Biol., 18, 222 (1968).

[10] S. W. May and N. N. Li, "The Immobilization of Urease Using Liquid-Surfactant Membranes," Biochem. Biophys. Res. Commun., 47, 5 (1972).

[11] T. M. S. Chang, in Recent Developments in Separation Science (N. Li, ed.), CRC Press, Cleveland, 1972.

[12] A. W. L. Jay and M. A. Edwards, "Mechanical Properties of Semipermeable Microcapsules," Can. J. Physiol. Pharmacol., 46, 731 (1968).

[13] A. W. L. Jay and K. S. Sivertz, "Membrane Resistance of Semipermeable Microcapsules," J. Biomed. Mat. Res., 3, 577 (1969).

[14] R. E. Sparks, R. M. Salemme, R. M. Meier, M. H. Litt and O. Lindan, "Removal of Waste Metabolites in Uremia by Microencapsulation Reactants," Trans. Am. Soc. Artif. Intern. Organs, 15, 353 (1969).

[15] S. N. Levine and W. C. LaCourse, "Materials and Design Consideration for a Compact Artificial Kidney," J. Biomed. Mat. Res., 1, 275 (1967).

[16] R. E. Sparks, O. Lindan, N. S. Mason, M. H. Litt, and P. M. Meier, "Removal of Uremic Waste Metabolites from the Gastrointestinal Tract by Encapsulated Carbon and Oxidized Starch," Trans. Am. Soc. Artif. Intern. Organs, 17, 229 (1971).

[17] P. V. Sundaram, "The Kinetic Properties of Microencapsulated Urease," Biochim. Biophys. Acta, 293, 1 (1973).

[18] D. L. Gardner, D. R. Falb, B. C. Kim, and D. C. Emmerling, "Possible Uremic Detoxification via Oral Ingested Microcapsules," Trans. Am. Soc. Artif. Intern. Organs, 17, 239 (1971).

[19] R. C. Boguslaski and A. M. Janik, "A Kinetic Study of Microencapsulated Bovine Carbonic Anhydrase," Biochim. Biophys. Acta, 250, 260 (1971).

[20] A. O. Mogensen and W. R. Vieth, "Mass Transfer and Biochemical Reaction with Semipermeable Microcapsules Containing Asparaginase," Ibid., 14, 663 (1972).

[21] T. Mori, T. Sato, Y. Matui, T. Tosa, and I. Chibata, "Preparation and Characteristics of Microcapsules Containing Asparaginase," Ibid., 14, 663 (1972).

[22] T. Mori, T. Tosa, and I. Chibata, "Enzymatic Properties of Microcapsules Containing Asparaginase," Biochim. Biophys. Acta, 321, 653 (1973).

[23] M. Shiba, S. Tomiika, M. Koishi, and T. Kondo, "Studies on Microcapsules. V. Preparation of Polyamide Microcapsules Containing Aqueous Protein Solution," Chem. Pharm. Bull., 18, 803 (1970).

[24] M. Kitajima and A. Kondo, "Fermentation without Multiplication of Cells Using Microcapsules That Contain Zymase Complex Muscle Enzyme Extract," Bull. Chem. Soc. Japan, 44, 3201 (1971).

[25] O. R. Zaborsky, Immobilized Enzymes, CRC Press, Cleveland, 1973.

[26] R. R. Mohan and N. N. Li, in Immobilized Enzymes (O. R. Zaborsky, ed.), CRC Press, Cleveland, 1973.

[27] J. C. W. Ostergaard and S. C. Martiny, "The Immobilization of β-Galactosidase through Encapsulation in Water-Insoluble Microcapsules," Biotech. Bioeng., 15, 561 (1973).

[28] T. M. S. Chang, F. C. MacIntosh, and S. G. Mason, "Semipermeable Aqueous Microcapsules," Proc. Can. Fed. Biol. Sci., 6, 16 (1963).

[29] T. M. S. Chang and F. C. MacIntosh, "Effects on Injected Semipermeable Aqueous Microcapsules," Ibid., 7, 58 (1964).

[30] T. M. S. Chang, "Asparaginase-Loaded Semipermeable Microcapsules for Mouse Lymphomas," Ibid., 12, 62 (1969).

[31] T. M. S. Chang, L. J. Johnson, and O. Ransome, "Semipermeable Aqueous Microcapsules: Nonthrombogenic Microcapsules with Herparin-Complexed Membranes," Can. J. Physiol. Pharmacol., 45, 70 (1967).

[32] T. M. S. Chang and M. J. Poznansky, "Semipermeable Aqueous Microcapsules (Artificial Cells): Permeability Characteristics," J. Biomed. Mat. Res., 2, 187 (1968).

[33] T. M. S. Chang, A. Pont, L. J. Johnson and N. Malave, "Response to Intermittent Extracorporeal Perfusion through Shunts Containing Semipermeable Microcapsules," Trans. Am. Soc. Artif. Intern. Organs, 15, 163 (1968).

[34] T. M. S. Chang, "Clinical Potential of Enzyme Technology," Sci. Tools, 16, 34 (1969).

[35] T. M. S. Chang, "Removal of Endogenous and Exogenous Toxins by a Microencapsulated Adsorbent," Can. J. Physiol. Pharmacol., 47, 1043 (1969).

[36] T. M. S. Chang and N. Malave, "The Development and First Clinical Use of Semipermeable Microcapsules (Artificial Cells) as a Compact Artificial Kidney," Trans. Soc. Artif. Intern. Organs, 16, 141 (1970).

[37] T. M. S. Chang, A. Gonda, J. H. Dirks and N. Malave, "Clinical Evaluation of Chronic Intermittent or Short Term Hemoperfusions in Patients with Chronic Renal Failure Using Semipermeable Microcapsule (Artificial Cells) Formed from Membrane-Coated Activated Charcoal," Ibid, 18, 465 (1972).

[38] T. M. S. Chang, "Hemoperfusions over Microencapsulated Adsorbent in a Patient with Hepatic Coma," Lancet, ii, 137 (1972).

[39] T. M. S. Chang, "Semipermeable Microcapsules Containing Asparaginase: In-vitro and In-vivo Stability," Enzym J., 14, 95 (1973).

[40] T. M. S. Chang, "Biomedical Application of Artificial Cells: Routes of Administration," Biomed. Bioeng. J., 8, 334 (1973).

[41] T. M. S. Chang, J. F. Coffey, P. Barre, A. Gonda, J. H. Dirks, M. Levy, and C. Lister, "Microcapsule Artificial Kidney: Treatment of Patients with Acute Drug Intoxication," Can. Med. Assoc. J., 108, 429 (1973).

[42] M. J. Poznansky and T. M. S. Chang, "Comparison of Enzyme Kinetics and Immunologied Properties of Catalase Immobilized by Microencapsulation and Catalase in Free Solution for Enzyme Replacement," Biochem. Biophys. Acta, 334, 103 (1974).

[43] E. Siu Chong and T. M. S. Chang, "In-vivo Effects of Intraperitoneally Injected L-Asparaginase Solution and L-Asparaginase Immobilized within Semipermeable Nylong Plasma L-Asparagine Levels," Enzyme J., 18, 218 (1974).

[44] T. M. S. Chang and S. K. Loa, "Urea Removal of Urease and Ammonia Absorbents in the Intestine," Physiologist, 13, 70 (1970).

[45] T. M. S. Chang, "Semipermeable Microcapsules as Artificial Cells," Sci. J., 3, 62 (1967).

[46] T. M. S. Chang and M. J. Poznansky, "Semipermeable Microcapsules Containing Catalase for Enzyme Replacement in Acatalasaemic Mice," Nature (London), 218, 243 (1968).

[47] T. M. S. Chang, "The In-vivo Effects of Semipermeable Microcapsules Containing L-Asparaginase on 6C3HED Lymphosarcoma," Ibid., 229, 117 (1971).

[48] T. M. S. Chang, "Stabilization of Enzyme by Microencapsulation with a Concentrated Protein Solution or by Microencapsulation Followed by Cross-Linking with Glutaraldehyde," Biochim. Biophys. Res. Commun., 44, 1531 (1971).

[49] T. M. S. Chang, "In-vitro Kinetics of Enzymes Immobilized by Microencapsulation," Biotech. Bioeng., 14, 520 (1972).

[50] T. M. S. Chang, A. Gonda, J. H. Dirks, J. F. Coffey, and T. Burns, "ACAC Microcapsule Artificial Kidney for the Long Term and Short Term Management of Eleven Patients with Chronic Renal Failure," Trans. Am. Soc. Artif. Intern. Organs, 18, 465 (1972).

[51] P. V. Sundarum, E. K. Pye, T. M. S. Chang, V. H. Edwards, A. E. Humphrey, N. O. Kaplan, E. Katchalski, Y. Levin, M. D. Lilly, G. Manecke, K. Mosbach, J. Porath, H. Weetall, and L. B. Wingrad, Jr., "Recommendations for Standardization of Nomenclature in Enzyme Technology," Biotech. Bioeng., 14, 15 (1972).

[52] T. M. S. Chang, "Immobilization of Enzymes, Adsorbents, or Both within Semipermeable Microcapsules (Artificial Cells) for Clinical and Experimental Treatments of Metabolite-Related Disorders," in Enzyme Therapy for Congenital Diseases (D. Bergsma, ed.), Williams and Wilkins, Baltimore, 1973, p. 66.

[53] T. M. S. Chang, J. F. Coffey, C. Lister, A. Stark, and E. Taroy, "Glutethimede, Methyprylon, and Methaqualone Clearance by the ACAC Microcapsule Artificial Kidney: In-vitro and in Patients with Acute Intoxication," Trans. Am. Soc. Artif. Intern. Organs, 19, 87 (1973).

[54] T. M. S. Chang and M. Migchelsen, "Characterization of Possible 'Toxic' Metabolites in Uremia and Hepatic Coma Based on the Clearance Spectrum of the ACAC Microcapsule Artificial Kideny," Ibid., 19, 314 (1973).

[55] T. M. S. Chang, "Enzymes Immobilized by Microencapsulation: Preparation and Biomedical Applications," in Insolubilized Enzymes (M. Salmona, C. Saronia, and S. Garattini, eds.), Raven, New York, 1973.