resulted in a further reduction of the *p*H to 3. Stirring of the combined solution at room temperature resulted in the formation of a precipitate in the form of a resinous, tacky composition which settled. The supernatant liquid was decanted, leaving the precipitated material. This material upon heating at a temperature of 38° to 93°C converted to a hard, brittle composition which broke with conchoidal fracture. The material was soluble in acetone, ethyl acetate, alcohols, and similar solvents. The composition also degraded when the material was placed in water.

Acids that have been polymerized include the herbicides 2,4-D, 2,4,5-T, Silvex, Dicamba, and Picloram (*1*). Others, without appreciable biological activity, include benzoic acid, acetic acid, stearic acid, salicylic acid, tartaric acid, and adipic acid. The rate of degradation can be decreased by heating the polymer, which increased its molecular weight and its hardness. The rate of degradation can also be controlled by varying the surface-to-volume ratio. Hydrophobic acids, such as stearic acid, slow the rate at which degradation occurs.

Initial emphasis has been placed on herbicidal uses for the polymers. Pollution of water supplies commonly accompanies the application of herbicides at useful concentrations. Rain or irrigation water washes away much of the herbicide; hence an overapplication by an order of magnitude is required for effective control. Furthermore, several additional applications per year are needed to maintain the herbicide concentration at an effective level. Polymerized 2,4-D and iron at a rate of 1 pound/acre (1.12 kg/ha) has given exceptional control of weeds in field tests on grazing lands in Kansas, and the control continues for more than 1 year. In other tests control of ironweed and Canadian thistle has been achieved. Further, no evidence for migration of herbicide was found, despite the sloping terrain and heavy rains (*4*).

The increase in efficiency for control of resistant plant species suggests that the concentration of herbicide at the root level is enhanced. This may be due in part to the hydrous ferric oxide gel, which minimizes leaching of the herbicide. Some applications were made from an acetone solution, and others were coated onto granules of diatomaceous earth which were then scattered onto the ground.

In the management of grazing land it is essential that the herbicide not be ingested by animals. The use of granules is an effective way of controlling herbicide ingestion, as they fall to the ground.

Preemergent soil treatment with a wide variety of herbicides and other agents is now possible with the advent of controlled release. The slow release of herbicides has been coupled with beneficial fertilization. Deliquescent 10-30-0 fertilizer prills (*1*) have been coated with polymer, and the fertilizer release was spread over several weeks.

These polymers are expected to improve the effectiveness of defoliation techniques. When sprayed as a solute in an organic solvent, the defoliant forms a sticky resin which clings to foliage. Even heavy rain does not appreciably wash it off. Hence, it is expected to stay where it is placed and do its job, with minimium runoff pollution of water supplies. It is expected that the amount of defoliant required can be reduced by an order of magnitude, with corresponding savings in money, time, and ecological upset.

Medical applications can also be expected. Benign polymeric formulations will be sought as encapsulating materials for pharmaceuticals, such that a slow release can be achieved by way of an implant.

These polymers have appreciable structural strength. As packaging for dry materials, or, with liners, for aqueous materials, the water-degradability feature is highly desirable. As an alternate to the use of nondegradable liners, a local cross-linking at exposed surfaces may impart an extremely slow rate of degradation. When the container is broken, the more readily degradable material will be exposed to moisture.

This wide class of water-degradable polymers offers great promise for the controlled release of herbicides, insecticides, nematocides, slimicides, fungicides, rodenticides, defoliants, and pharmaceuticals. They may also find utility in the packaging industry, since they speedily break down after use.

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1. 2,4-D: (2,4-dichlorophenoxy)acetic acid; 2,4,5-T: (2,4,5-trichlorophenoxy)acetic acid; Silvex: 2-(2,4,5-trichlorophenoxy)propionic acid; Dicamba: 2-methoxy-3,6-dichlorobenzoic acid; Picloram: 4-amino-3,5,6-trichloropicolinic acid; 10-30-0 fertilizer contains 10 percent (by weight) nitrogen, 30 percent phosphoric acid, and no potash.
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Immunoglobulin M Heavy Chain Disease: Intracellular Origin of the Mu Chain Fragment

Abstract. *Cells obtained from a patient with mu heavy chain disease synthesize a mu heavy chain fragment with a molecular weight of 55,000. The fragment is detected intracellularly after short labeling times and then is assembled inside the cell and secreted as a disulfide-linked polymer.*

Mutations in bacteria which result in the production of incomplete polypeptide chains have provided models for elucidating the mechanisms governing protein synthesis. Similar aberrations are notably rare in mammalian cells. Therefore the possible occurrence of such an abnormality of human immunoglobulin synthesis is of great interest. The secretion and structure of naturally occurring fragments of the γ and α heavy chains have already been described (*1*).

The serum of a patient with chronic lymphocytic leukemia was found to contain both a fragment of the immunoglobulin M (IgM) μ heavy chain and free kappa light chains (*2*). We now report the results of our study of the intracellular origin of the μ fragment, the kinetics of its synthesis, its assembly into polymers, and its secretion. The cellular origin of the fragment and the structure of light chains have been described (*3*).

Immunoglobulin-producing cells were obtained when the patient required surgery for repair of a pathologic fracture of the right femur. The cells were immediately suspended in Eagle's minimal essential medium except that it contained only one-twentieth the usual con-

centrations of valine, threonine, and leucine. The cells were washed twice in the same medium, and the contaminating red cells were lysed by suspending the cells in hypotonic buffer for 60 seconds; the salt concentration was then adjusted to isotonicity. The remaining cells were washed in the same medium and resuspended at a concentration of 5×10^6 cells per milliliter. After ^{14}C-labeled valine, threonine, and leucine were added to the medium, the cells were incubated at 37°C in a bath which was shaken mechanically. At various times, samples of cells and medium were removed, chilled to stop protein synthesis, and centrifuged at 1500 rev/min for 8 minutes at 4°C. The supernatants containing the secreted material were removed and prepared for analysis. The cells were then washed, and the remaining contaminating red cells were lysed. After a second washing, the cells were resuspended in isotonic phosphate buffer, *p*H 7.2, to which the detergent Nonidet P-40 (0.5 percent final concentration; Shell Chemical Co.) was added, and allowed to stand for 15 minutes at 4°C. This detergent dissolves the outer cell and cytoplasmic membrane but leaves the nuclei intact (*4*). The cells were centrifuged at 40,000 rev/min for 30 minutes; the pellets containing nuclei and ribosomes were discarded while the cytoplasm was prepared for analysis. One portion of each sample of cytoplasm was made 0.03*M* with iodoacetamide (recrystallized), 0.01*M* with sodium phosphate buffer (*p*H 7.2) and 2 percent with sodium dodecyl sulfate (SDS); the adjusted samples were then placed in a boiling water bath for 1 minute (*5*).

Other portions were precipitated with antiserums specific for μ or light chain determinants. The precipitates were washed twice with cold, phosphate-buffered saline and then dissociated in iodacetamide and SDS as above. Some of the dissociated cytoplasmic immune precipitates were subsequently reduced and alkylated. All the dissociated samples were analyzed on acrylamide gels (20 cm) containing 0.1 percent SDS and 0.1*M* sodium phosphate, *p*H 7.2. The top 2 cm of each gel was 3.5 percent, the remaining 18 cm was 5 percent. Each sample was subjected to electrophoresis with tritium-labeled material secreted by the mouse myeloma MPC-11 as a marker. The antiserums to μ and κ chains were similar to those used previously (*6*).

The electropherogram of cytoplasmic

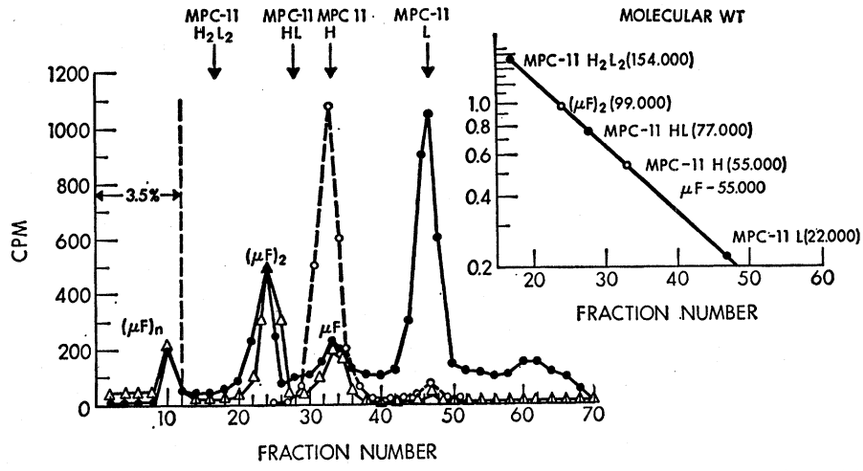

Fig. 1. Electropherograms of cytoplasm obtained after 17 minutes of incubation with ^{14}C-labeled amino acids. Electrophoresis is from left to right. (Solid circles) Total cytoplasm dissociated in 2 percent SDS in the presence of 0.03*M* iodoacetamide. (Open trianges) Cytoplasm precipitated with antiserum directed against human IgM. (Open circles) Cytoplasm precipitated with antiserum to IgM, dissociated in SDS, and then reduced (0.15*M* mercaptoethanol) and alkylated with iodoacetamide. All samples were subjected to electrophoresis with tritiated, secreted immunoglobulin G from MPC-11 (*10*). The positions of the markers are denoted by the arrows. The molecular weights of intracellular immunoglobulins are plotted in the insert (*13*); CPM, counts per minute.

material obtained after 17 minutes of continuous labeling is shown in Fig. 1. The immediately dissociated cytoplasm contains four main peaks of radioactivity. The first of these remains in the 3.5 percent gel, and its molecular weight cannot be accurately estimated in this two-phase gel system. However, it is larger than 350,000 because smaller proteins can enter a 5 percent acrylamide gel. It is precipitated by antiserum directed against μ chains and does not seem to contain light chains because no light chains are released after reduction and alkylation (see below). It represents the largest intracellular polymer unit of the basic synthetic μ chain fragment (μF) and has been designated $(\mu F)_n$. The second peak, $(\mu F)_2$, has a molecular weight of approximately 100,000; it represents the bulk of the cytoplasmic protein that is precipitable by antiserum to μ chain and is present after 17 minutes of labeling. The third peak, μF, is the primary polypeptide produced by these cells. The molecular weight of this subunit is 50,000 to 55,000, and its migration on the acrylamide gel coincides with that of a reduced and alkylated γ2b heavy chain secreted by MPC-11, an indication that both are the same size. The fourth peak, seen only in the immediately dissociated cytoplasm, is not precipitable with antiserum directed toward μ determinants and on coelectrophoresis it migrates with MPC-11 light chains. The material is also precipitable with antiserum which reacts with light chains (not shown in Fig. 1).

Reduction and alkylation of the cytoplasm precipitated by antiserum to μ chain results in a single peak that migrates in the same position as the μF fragment. No material migrating with L chains is seen. Thus, no more than 5 percent of the μ chain fragment molecules can be associated with light chains, and all the intracellular material which can be precipitated with antiserum to μ chain can be reduced to a polypeptide which corresponds in size to the native μF fragment.

It is evident from Fig. 2 that μF is assembled into the dimer $(\mu F)_2$ and subsequently assembled into the $(\mu F)_n$ polymer. Although the time required for assembly cannot be estimated from this type of study, it would appear that the proportion of intracellular material that is precipitable by antibody to μ chain and that migrates as $(\mu F)_n$ increases with time. The intracellular polymer appears to be smaller than the 19*S* IgM synthesized in cells that secrete intact macroglobulin (*7*).

An electropherogram of secreted material precipitated with antiserum obtained after 60 minutes of labeling is shown in the insert of Fig. 2C. Almost all of the material remains in the 3.5 percent acrylamide portion of the gel, indicating that it is a covalently linked large polymer bearing μ chain antigenic determinants. Secreted material obtained at additional intervals during labeling shows similar electrophoretic patterns.

Reduction and alkylation of secreted material and of the protein isolated from the patients' serum also fails to reveal light chains covalently bound to the μ fragment.

Earlier studies carried out with cells producing α and γ chain fragments from patients with α and γ heavy chain disease indicated that the fragments were not products of extracellular catabolism, thus confirming studies performed in vivo on the original patient with heavy chain disease (*1, 8*). Our study of human cells which secrete a μ chain fragment exclude the existence of a normal chain or polymer which is sequentially degraded to a smaller fragment after the μ chain is released from the ribosome. Instead, these experiments indicate that the fragment is the primary intracellular synthetic product and that it is a precursor of the intracellular and secreted polymer. Our studies show that the primary synthetic subunit (μF) has a molecular weight of about 55,000 and readily polymerizes into a group of larger polymers, all of which lack covalently bound light chains. The relative amounts of polymeric species seen at various intervals represent an ordered pattern of assembly, $\mu F \rightarrow (\mu F)_2 \rightarrow (\mu F)_n$, which is analogous to that seen in some cell populations obtained from patients producing intact macroglobulins, that is, $\mu L \rightarrow (\mu L)_2 \rightarrow [(\mu L)_2]_5$ (*7*). From the structure of native macroglobulin which appears to consist of a circular pentamer of five subunits each composed of two light (L) and two heavy (H) chains (H_2L_2) (*9*), we think it possible that in $(\mu F)_n$, n is 10 and can be expressed as $[(\mu F)_2]_5$.

This patient differs from others with γ and α heavy chain disease in producing both the fragment and free light chains. Since the kinetic studies presented here represent the average behavior of a mixed cell population, no conclusions can be drawn concerning the relative rates of synthesis of the two polypeptides, nor can it be stated with certainty whether they are synthesized in the same or different cells. However, immunofluorescence studies have resulted in the identification of cells containing both μ and κ chains, an indication that there may be a defect in assembly related to the structure of the abnormal μ chains (*3*).

In some tumors, and in the lymph node of hyperimmunized rabbits, light chains may play a role in both the release of heavy chain from polysomes and the secretion of the assembled protein (*10, 11*). In patients with γ and α chain disease, and now in this patient with μ chain disease, it would appear that covalently bound light chains are not necessary for the secretion of heavy chain fragments. It is still uncertain whether covalent interaction between H and L is necessary for the secretion of intact heavy chains.

Our data reveal that an abnormality of heavy chain synthesis is not always associated with an inability to produce light chains; hence, cells from patients with γ and α chain disease, which produce heavy chain fragments but not light chains, may have two distinct disorders of synthesis. Production of aberrant heavy chains by cells also synthesizing intact light chains apparently occurs in several mouse myelomas (*11, 12*).

The synthesis in vivo and secretion of a peptide corresponding to the constant portion of the heavy chain has been cited as evidence in favor of two genes coding for the complete heavy chain (*8*). Our data establish the intracellular synthetic origin of the heavy chain fragment, but they do not reveal whether this fragment represents an incomplete polypeptide resulting from a deletion or a terminator mutation that is followed by reinitiation within a single heavy chain gene, or whether it is the intact protein product of the entire second cistron of a molecule normally coded by two genes.

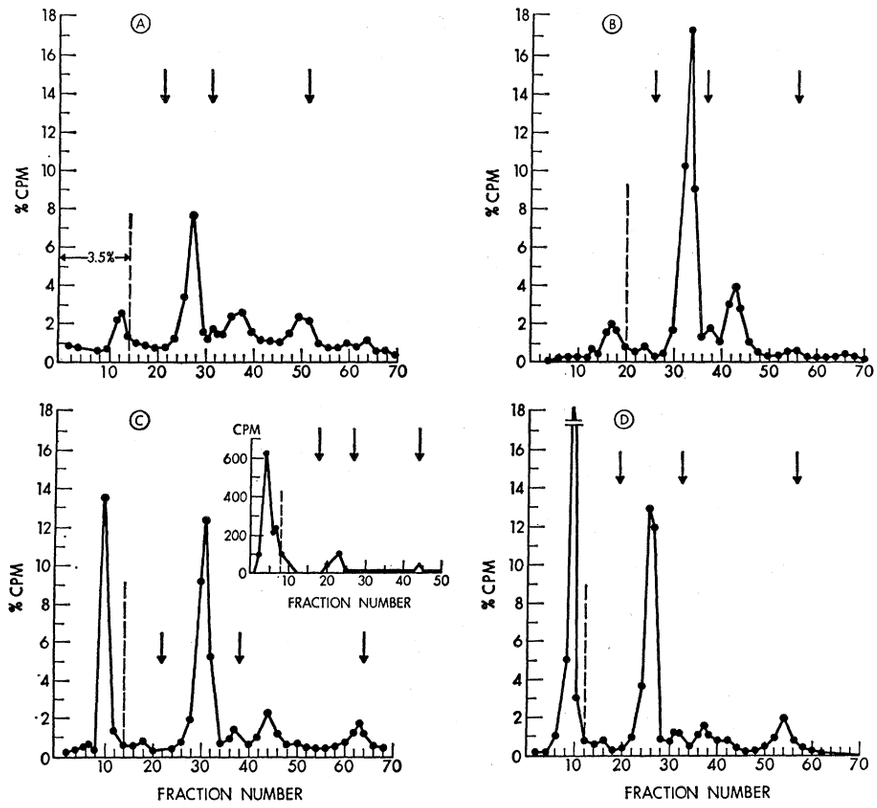

Fig. 2. Electropherograms of cytoplasm precipitated by antibody to μ chains obtained after (A) 5 minutes, (B) 10 minutes, (C) 60 minutes, and (D) 150 minutes of incubation. The small peak of radioactivity corresponding to light chain on each gel probably represents channel overlap from the marker proteins. The arrows represent the H_2L_2, HL, and L chain proteins of MPC-11. The insert in C shows the pattern of the immunologically precipitated, secreted material seen after 60 minutes of incubation; CPM, counts per minute.

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Biosynthesis of Vitamin B_6: Incorporation of Three-Carbon Units

Abstract. *Pyridoxol, one of the forms of vitamin B_6, is derived from three glycerol units. One of these is incorporated by way of pyruvate as a two-carbon fragment at the oxidation level of acetaldehyde. The other two glycerol units are incorporated intact, possibly by way of triose phosphate.*

Although it is well recognized that vitamin B_6 is biosynthesized by plants, algae, and most microorganisms, little is known of the pathways involved (*1*). We now offer definite evidence concerning the primary precursors of pyridoxine (that is, pyridoxol), one of the forms of the vitamin.

The major obstacle in the investigation of the biosynthesis of vitamin B_6 is the minute concentration (40 to 200 ng per milligram of dry cell material) (*2*) at which it is present in systems that produce it. Success in biosynthetic tracer studies of the origin of B_6 is thus contingent on a high radiochemical yield in the incorporation of radioactive tracer, such that the specific activity of the product, even after manyfold dilution with inactive carrier, is still high enough for degradation studies. The incorporation of activity from ^{14}C-labeled substrates into pyridoxamine in the yeasts *Candida utilis* (*3, 4*) and *C. albicans* (*5*) was insufficient for this purpose. Nonrandom incorporation of activity from [2-^{14}C]glycerol (*6*) and from DL-[2-^{14}C]aspartate (*7*) into pyridoxamine was demonstrated in a bacterial strain isolated from soil. Partial degradation indicated that little of the activity was present at the aminomethyl and the hydroxymethyl carbon atoms (C-4′ and C-5′). The sites of labeling were not further investigated, however.

We have examined the biosynthesis of pyridoxol in a mutant of *Escherichia coli* (*8*), which is blocked between pyridoxol and pyridoxal (*9*). When deprived of exogenous pyridoxal, this mutant generates pyridoxol and excretes it into the culture medium. Cultures of the mutant were grown to exponential phase in a minimal medium (*9*) supplemented with pyridoxal. The cells were then harvested, washed free of pyridoxal, and resuspended in pyridoxal-free minimal medium to which labeled substrate had been added. Incubation was resumed and continued for 5 to 6 hours, at which time labeled pyridoxol hydrochloride was isolated from the medium by ion exchange (Dowex 50 × 8) and thin-layer (silica gel G) chromatography, after addition of inactive carrier (Table 1).

The labeled samples of pyridoxol hydrochloride were purified to constant radioactivity by repeated crystallization and vacuum sublimation. Kuhn-Roth oxidation then yielded acetic acid, isolated as the α-naphthylamide (*10*), from C-2 and the adjacent C-methyl group (C-2′). Degradation of the acetic acid by the Schmidt reaction in turn gave the C-methyl group as methylamine, isolated as the *N*-dinitrophenyl derivative. The specific activities of the samples of pyridoxol and the corresponding degradation products are presented in Table 2.

Pyridoxol, obtained from the cultures incubated with [1-^{14}C]glycerol (experiments 1 and 2), contained approximately one-fifth of its label in the two-carbon unit (C-2′, C-2) (acetic acid), irrespective of whether glycerol or glucose had served as the general carbon source. Since virtually all the activity of this acetic acid was located at the C-methyl group (C-2′) (methylamine), distribution of label from [1-^{14}C]glycerol was nonrandom (*11*) (experiment 2). It may be inferred from this result that five of the eight carbon atoms of pyridoxine might be ultimately derived from the terminal carbon atoms of glycerol.

In an attempt to define more precisely the relation to pyridoxol of three-carbon compounds related to glycerol, incorporation of label from pyruvic acid was investigated. All activity of pyridoxol from [2-^{14}C]pyruvate (experiment 5) and from [3-^{14}C]pyruvate (experiment 3) was found in the Kuhn-Roth acetate (C-2′, C-2), whose C-methyl carbon (C-2′) is supplied entirely by the C-methyl group of pyruvate (experiment 4). The two-carbon unit (C-2′, C-2) of pyridoxol is thus derived specifically from the methyl and the carbonyl carbon atoms of pyruvate.

To test whether the carboxyl carbon of pyruvate serves as the source of C-3 of pyridoxol, that is, whether an intact pyruvate moiety yields the three-

Table 1. Incorporation of three-carbon units into pyridoxol. The general carbon source in experiment 1 was glucose (0.2 percent, weight to volume). In all other experiments the general carbon source was glycerol (0.2 percent, weight to volume). The culture volume in experiment 6 was 2 liters. In all other experiments it was 1 liter.

Expt. No.	Substrate*	Nominal		Carrier added (g)	Pyridoxol hydrochloride specific activity (10^4 count min^{-1} $mmole^{-1}$)
		Total activity (mc)	Specific activity (mc/mmole)		
1	[1-^{14}C]Glycerol	0.1	15.4	0.04	1.78 ± .05
2	[1-^{14}C]Glycerol	0.5	0.023†	0.12	2.38 ± .06
3	[3-^{14}C]Pyruvic acid	0.1	35.6	0.04	1.20 ± .03
4	[3-^{14}C]Pyruvic acid	0.1	35.6	0.09	0.54 ± .01
5	[2-^{14}C]Pyruvic acid	0.1	31.7	0.04	0.72 ± .02
6	[1,3-$^{14}C_2$]Pyruvic acid‡	0.3	30.8	0.08	1.05 ± .02
7	[2-^{14}C]Acetic acid	0.1	54.7	0.04	0.36 ± .01

* Labeled compounds were obtained from Amersham/Searle (experiments 1, 3–7) and from Commissariat à l'Energie Atomique (experiment 2). † Obtained by mixing [1-^{14}C]glycerol, nominal specific activity 10 mc/mmole (4.6 mg), with inactive glycerol (2 g). ‡ Obtained by mixing [1-^{14}C]-pyruvic acid (0.15 mc, 27.2 mc/mmole) and [3-^{14}C]pyruvic acid (0.15 mc, 35.6 mc/mmole). For determination of distribution of activity, see Table 2, last note (§).