The Translation of Messenger RNA for Mouse Immunoglobulin Light Chains in Living Frog Oocytes

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Messenger RNA for mouse immunoglobulin light chains is translated into proteins resembling light chains when injected into living frog oocytes. Frog oocytes can therefore translate purified messenger RNA of a kind which normally promotes protein synthesis on membrane-bound polysomes.

Frog oocytes injected with 9s RNA purified from rabbit reticulocytes synthesize globin very efficiently (Lane, Marbaix and Gurdon, 1971). It is not known that frog oocytes can translate purified messenger RNA for that class of proteins which, in contrast to haemoglobin, is exported from cells where they are formed, and which is synthesized on membrane-bound polysomes. An example of such proteins is the immunoglobulin light chain synthesized in mouse myeloma cells (MOPC 41) (Scherr and Uhr, 1970; Lisowska-Bernstein _et.al._, 1970). Stavnezer and Huang (1971) have prepared messenger RNA from these cells and translated it in a rabbit reticulocyte cell-free system. We describe here the synthesis of light chain proteins in frog oocytes injected with myeloma mRNA. Since our experiments were initiated, Stevens and Williamson (1972), using these methods, have obtained antibody precipitable proteins from myeloma RNA-injected _Xenopus_ oocytes. They found that these proteins comigrated, electrophoretically, in SDS gels with immunoglobulins synthesized in myeloma cells. To prove that this messenger RNA is correctly translated, we have carried out a tryptic peptide analysis of the proteins synthesized in oocytes after injection of myeloma RNA.

K-41 mRNA was prepared as reported by Stavnezer and Huang (1971). The procedures for preparing, injecting, incubating, and labelling

oocytes of Xenopus laevis were those described by Gurdon, Lane, Woodland and Marbaix (1971). Gel electrophoresis was carried out according to the procedure of Laemmli (1970), and the analysis of tryptic peptides according to Stavnezer and Huang (1971).

Each sample of oocytes was homogenised in one ml of PBS (0.14M NaCl, 1.5mM KH_2PO_4, 2.7 mM KCl, 8.1 mM Na_2HPO_4) and centrifuged for 5 minutes at 3000g. A portion of the supernatant was removed for total acid precipitable counts. Sufficient purified urinary K-41 protein was added as carrier to the egg supernatants to reach the equivalency point with 0.2 ml of antiserum, as determined by quantitative precipitin assays. Precipitations were performed in the presence of 3% Triton X-100 (Packard) and 0.01M EDTA at pH 7.9. The antibody precipitates were pelleted at 1500g for 15 minutes and washed twice in cold PBS, once in 10% TCA for 15 minutes at 85°C, and once each in ethanol and ether. The precipitates were collected on GF/C filters, and counted. Antibody to K-41 precipitates 4-6 times more of the TCA-insoluble counts in supernatants of oocytes which had been injected with K-41 mRNA than in supernatants of control oocytes given injection medium (see Table 1). It is not known whether the background label observed in control oocytes represents endogenous oocyte proteins cross reactive with K-41 or simply trapping of radioactivity in the precipitates.

Sephadex fractionation and gel electrophoresis were carried out on samples prepared from another set of message-injected oocytes, labelled with ^{3}H-leucine. The washed antibody-precipitate was dissolved in 1 ml of 6.7M GuCl, 0.1M Tris-HCl pH 8, and dialyzed against 0.05M glycine-HCl pH 2.4 to dissociate the antibody-antigen complex. This procedure yields an approximately 60% dissociation of antibody from antigen. Carrier urinary K-41 protein (2 mg) was added to the dialyzed sample and it was chromatographed on a Sephadex

G100 column equilibrated in 0.05M glycine-HCl. 2 ml fractions were collected and a portion of each was counted. As seen in Fig. 1, the radioactivity from oocytes injected with mRNA is eluted as three peaks, corresponding to the undissociated antibody-antigen complex, the dimer form, and the monomer form of K-41. Saline-injected control oocytes did not contain a peak of radioactivity which eluted in any of these three regions. For gel electrophoresis, the sephadex fractions referred to above were pooled as three radioactivity peaks from the separation shown in Fig. 1A, and as one peak (fractions 15-39) as seen in Fig. 1B. Each pooled sample was lyopholized and dissolved in sample buffer containing 2% SDS and 5% β-mercaptoethanol, as described by Laemmli (1970); electrophoresis was run at 4^oC. The gels were fixed, stained, and destained. The position of the K-41 carrier proteins was measured and the gels frozen and sliced; each slice was dissolved and counted. The results of electrophoresis are shown in Fig. 2. Little if any of the radioactivity of control oocytes comigrates with authentic urinary K-41; in contrast, all the samples from mRNA-injected oocytes contained proteins which comigrated with K-41.

For the analysis of tryptic peptides, message-injected oocytes were labelled with ^{14}C-leucine. Oocyte homogenates were precipitated with antibody, and passed through Sephadex columns as described above. Material from the Sephadex column was pooled as two samples, consisting of the excluded antibody-antigen peak, and the combined monomer and dimer regions. Authentic tumor K-41, labelled with ^{3}H-leucine, was added as marker and analysed simultaneously with the oocyte products. Proteins were reduced with 0.05M dithiothreitol, alkylated with 0.113M iodoacetic acid (Cebra et.al., 1968) and digested for 18 hours at 37^oC with trypsin which had been pre-treated with L-1-Tosyl(amido-2-phenyl) ethyl chloromethyl ketone (Kostka and Carpenter, 1964). The digested

sample, dissolved in 3% pyridine, was applied to a 6mm x 120cm column of 'Dowex 1-X2' equilibrated in 3% pyridine. Following elution from the column, samples of each fraction were counted directly in Triton fluor. Material from the excluded antibody-antigen peak, as well as from the combined monomer and dimer regions, gave a similar distribution of peptides, like that shown in Fig. 3. There is a close resemblance between the general distribution of oocyte-synthesized and myeloma cell-synthesized radioactivity.

These results demonstrate a resemblance between oocyte-derived light chains and light chains synthesized in myeloma cells, as judged by antibody precipitation, Sephadex chromatography, SDS acrylamide gel electrophoresis, and by the Dowex fractionation of materials released by trypsin digestion. Comparable oocytes injected with saline solution but with no mRNA yielded no antibody-precipitable material which resembled myeloma light chains by any of these criteria. Our results therefore confirm and extend the observations of Stevens and Williamson (1972). Myeloma mRNA is the first example of a type of message which normally functions on membrane-bound polysomes to have been demonstrated to be translated in living frog oocytes. In view of the additional fact that mRNAs for rabbit, mouse, and duck globins, (Marbaix and Lane, 1972; Lane _et.al._, 1973), for calf αA_2 crystallin (Berns _et.al._, 1972), and for EMC virus (Laskey, Gurdon and Crawford, 1972) are all translated in oocytes, it is likely that oocytes can translate all kinds of vertebrate messenger RNAs. These results with myeloma messenger RNA much strengthen the view that _Xenopus_ oocytes contain translational systems which are neither cell-type nor species specific.

The concentration of messenger RNA which we have used (50 ng per cell) resulted in only 2-3% of the oocyte's protein being of myeloma-type, on the assumption that endogenous synthesis was not

reduced (as it appears not to be from the ratio of acid insoluble to acid soluble counts in RNA-injected and control oocytes). This amount of stimulation is ten times lower than that obtained by a saturating concentration of haemoglobin mRNA (Moar et.al., 1971). This difference could very well be due to the relative purity of the messenger RNA preparations, since the light chain mRNA used in these experiments was much less pure than the haemoglobin mRNA referred to.

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TABLE 1

Synthesis of myeloma-like protein by frog oocytes injected with myeloma RNA

Solution injected	Label	Cts/min total protein	Cts/min precipitated by antibody	Antibody precipitate as % of total protein	Stimulation due to injected RNA
RNA		281,900	7,661	2.4	
	^{3}H-threonine				4.0
Saline		314,120	1,744	0.6	
RNA		3,579,680	136,963	3.83	
	^{3}H-serine				5.4
Saline		3,088,930	21,825	0.71	

Each oocyte was injected with 50mμl of 9-13S myeloma RNA at approximately 1 mg/ml. Each sample contained 50 oocytes which had been incubated in label for 17 hours at 19°C. After homogenisation, a portion of the supernatant was precipitated with trichloroacetic acid (TCA) and collected on Whatman GF/C filters to determine the incorporation of radioactivity into total protein. The remaining portion was incubated with K-41 carrier protein and rabbit anti-K41 antiserum for 30 minutes at 37°C, and subsequently at 4°C for 16 hours (^{3}H-thronine labelled oocytes) or 47 hours (^{3}H-serine labelled oocytes). The antibody precipitation assays were performed as described above.

Figure 1.

Sephadex G-100 analysis

Two groups of 70 oocytes from the same frog were injected with 70 ng of mRNA or injection medium and incubated for 24 hours in 2 mC/ml ^{3}H-leucine. After homogenisation and centrifugation, the supernatants were antibody precipitated; the precipitates were dissociated by dialysis against 0.05M glycine-HCl pH 2.4, and run on a 1.5 x 72cm Sephadex G-100 column, in the same glycine-HCl buffer. A, mRNA-injected; B, control.

Figure 2.

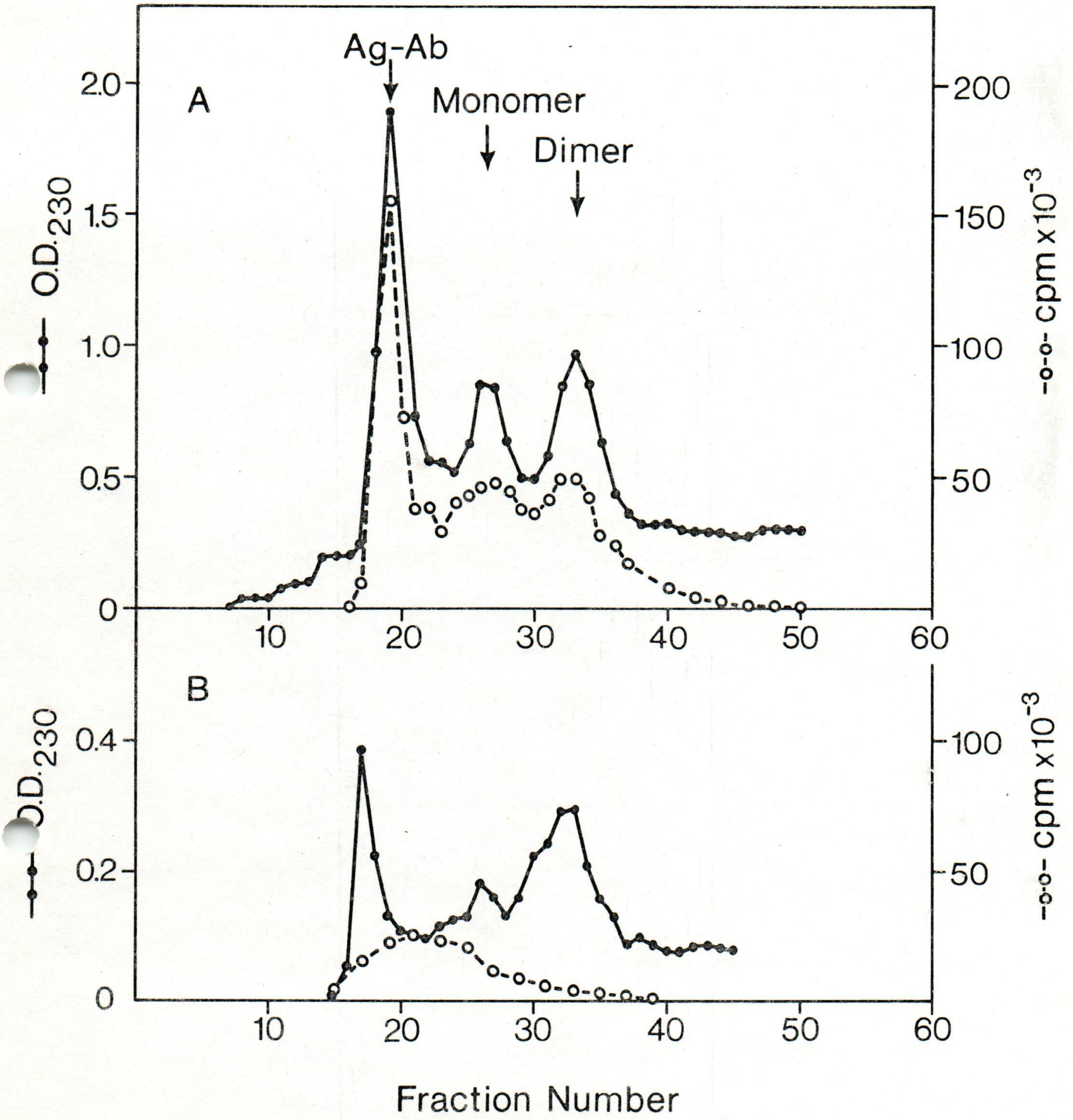
SDS acrylamide gel electrophoresis of protein separated on Sephadex G-100

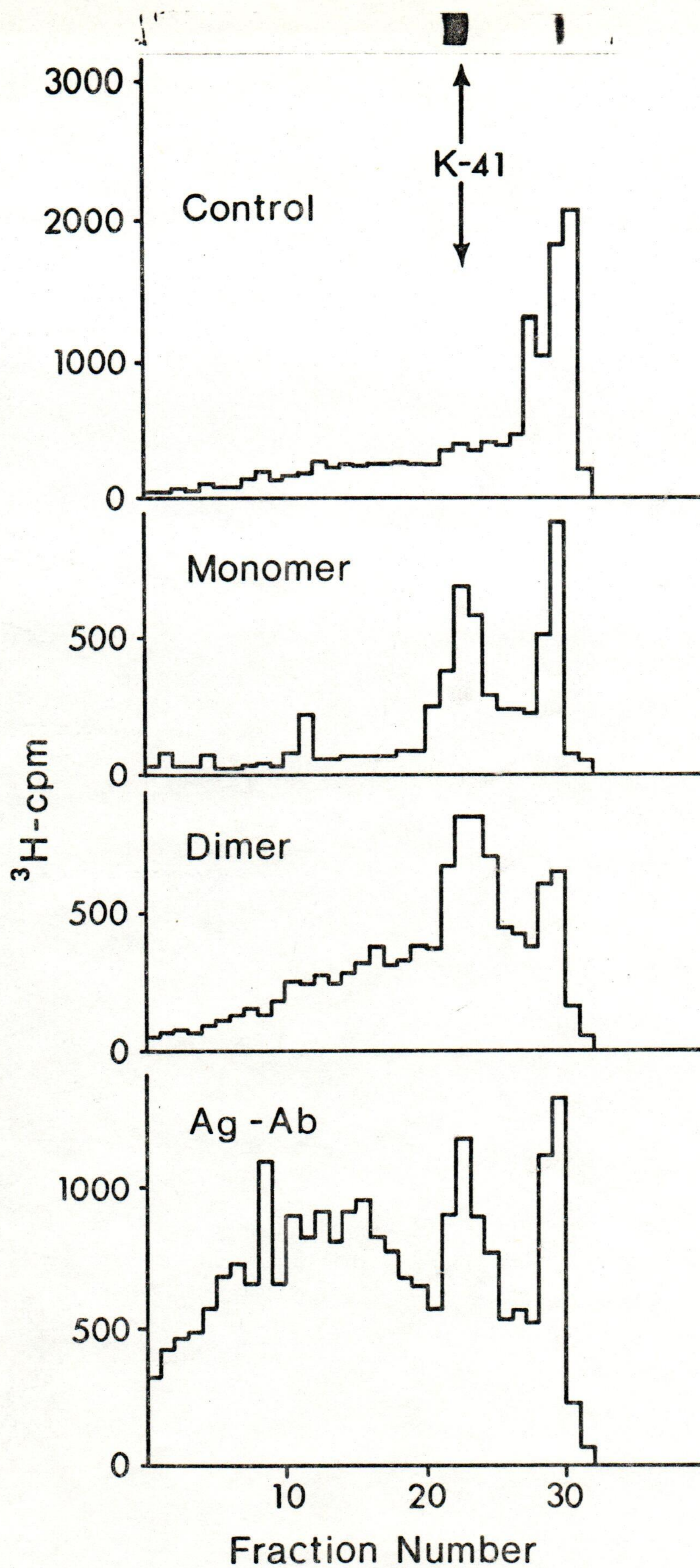
10% polyacrylamide-SDS gels were used with a 3% stacking gel, according to Laemmli (1970). The N,N'-bis-acrylamide in the gels was replaced by 0.8% ethylene diacrylate. Samples were heated in a boiling water bath for 1 minute prior to loading on to the gel. After electrophoresis, the gels were fixed in 50% TCA, stained in Coomassie Blue, and destained in 7% acetic acid. The gels were cut into 5mm sections and incubated at 60°C in 1 ml of 0.1M NaOH until the slices dissolved. The samples were acidified with acetic acid and counted in Triton X-100 fluor.

Figure 3.

Analysis of tryptic peptides

Dowex 1-X2 resin was prepared by using the fines from the Bio-Rad product AG1-X2. The resin was suspended in water and allowed to settle for 10 minutes and the fines were decanted and saved; the process was repeated four or five times. The column was maintained at 37°C with a water jacket. The peptides were eluted with a gradient of 3% pyridine (260 ml) to 0.1M pyridine, 0.05M acetic acid (130 ml). This was followed by a gradient of 0.1M pyridine, 0.05M acetic acid (150 ml) to 1M acetic acid (75 ml). The flow rate was maintained at 20 ml/hour.


Ag-Ab
Monomer
Dimer
A
B
O.D.230
cpm x 10-3
Fraction Number
2.0
1.5
1.0
0.5
0
200
150
100
50
0.4
0.2
10
20
30
40
50
60



K-41
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Dimer
Ag - Ab
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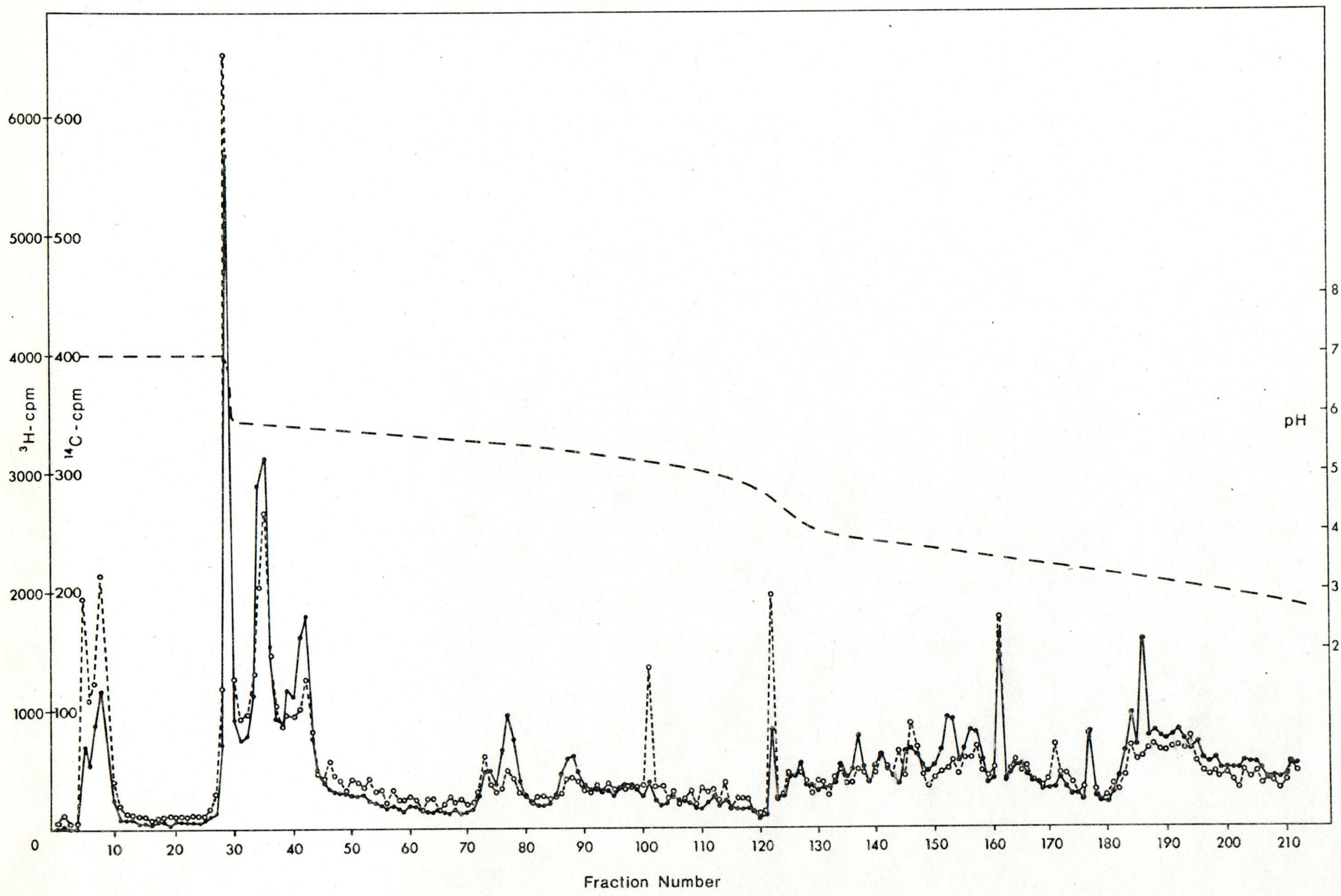
2000
1000
500
0
^{3}H-cpm
10
20
30
Fraction Number



^{3}H-cpm
^{14}C-cpm
6000 600
5000 500
4000 400
3000 300
2000 200
1000 100
0
pH
8
7
6
5
4
3
2
10
20
30
40
50
60
70
80
90
100
110
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140
150
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170
180
190
200
210
Fraction Number