IgM antibody to *Salmonella typhosa* "O" antigen fixes complement so efficiently that "whole antiserum will behave like the 19*S* fraction" (*17*) suggests that generalizations concerning immunoglobulin classes based on the results of complement-dependent bactericidal tests should be made with caution.

The demonstration of natural antibodies to *E. coli* and *N. gonorrhoeae* in cord serums has bearing on the study of resistance to infection in newborns. The relative susceptibility of newborns to infection with Gram-negative bacteria might be explained by their deficiency of natural IgM bactericidal antibody (*4*). However, several observations suggest that IgM bactericidal activity is not the principal means of defense. Although virtually all newborn infants lack IgM bactericidins to an equal degree and all are exposed to a potentially pathogenic flora at birth, only a very small number succumb to infection with Gram-negative bacteria (*1*, *5*). The finding that the cord serums examined contained natural antibodies of the IgG class suggests that such IgG antibodies may contribute to the resistance demonstrated by most newborns to Gram-negative bacterial infections. Although IgG antibody may appear to be less reactive in bactericidal tests, it may perform a critical function within the host's antibacterial recognition system.

The current concept (*18*) that a sequential appearance of IgM and IgG antibodies occurs after immunization should be reexamined in the light of our finding that natural IgG antibody in humans may be present prior to immunization or symptomatic infection. The concept of a sequential immunoglobulin response has also been questioned in a study of antibodies produced by immunized rabbits (*19*).

In summary, immunofluorescence techniques can reveal important antibody activity which may elude detection by other methods. It appears that the natural humoral recognition systems for the heat-stable somatic antigens of Gram-negative bacteria are not limited to IgM antibodies alone and that a major portion of this natural antibody activity resides in the IgG class.

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Immunoglobulin Synthesis in vitro by Established Human Cell Lines

Abstract. *Several lines of human lymphoid cells derived from malignant lymphoma produce immunoglobulins in vitro. Immunoglobulins G(IgG, γG) are synthesized in two cell lines and immunoglobulin M (IgM, γM) in one.*

Continuous production in vitro of antibody or other immunoglobulins by human cells has not been reported. Until 1964, no continuous cultures of human lymphoid or plasma cells were available. Pulvertaft and Epstein and his associates first described propagation in vitro of lymphoid cells in suspension culture from malignant lymphomas of African children (*1*) and, subsequently, similar cell lines have been isolated in several other laboratories (*2*, *3*). The patients from whom these cells were derived had maglignant lymphomas with clinical and pathological features which Burkitt and O'Conor described in most cases of childhood lymphoma in Africa (*4*). These neoplasms are now referred to as "Burkitt's tumors." Dorfman and O'Conor *et al.* (*5*) have described similar tumors in American patients and from one of these, a young American woman, a cell line similar to the lines obtained from the African children has been isolated (*6*). The present studies were carried out with the cell line derived from this American patient, designated AL-2, and with three cell lines from African patients designated AL-1, EB-2, and Ogun (*7*). These lines were maintained in culture in a medium composed of 80 percent mixture No. 199 and 20 percent fetal bovine serum. After 48 hours of cell growth, the culture fluid freed from cells was concentrated 20-fold and examined by immunoelectrophoresis for the purpose of detecting any human immunoglobulins that might be present.

Human immunoglobulins were identified with antiserums specific for IgG, IgA, IgM, or IgD [that is, for polypeptide γ-, α-, μ-, and δ-chains (heavy) of human immunoglobulins] and for molecules with κ and λ light polypeptide chains (*8*). Antiserums were absorbed with fetal bovine serum and with bovine γ-globulin to remove cross-reacting antibodies that might impair detection of human immunoglobulin in the presence of the fetal bovine serum of the culture medium.

Immunoelectrophoresis of the concentrated incubation medium from line AL-2 indicates a relatively homogeneous immunoglobulin in the cathodal (slow gamma) region (Fig. 1). No immunoglobulin was detected in the medium from AL-1 cells. Biosynthetic techniques confirmed the synthesis of immunoglobulin by AL-2 cells and the absence of immunoglobulin formation in the AL-1 line.

The method for detection of im-

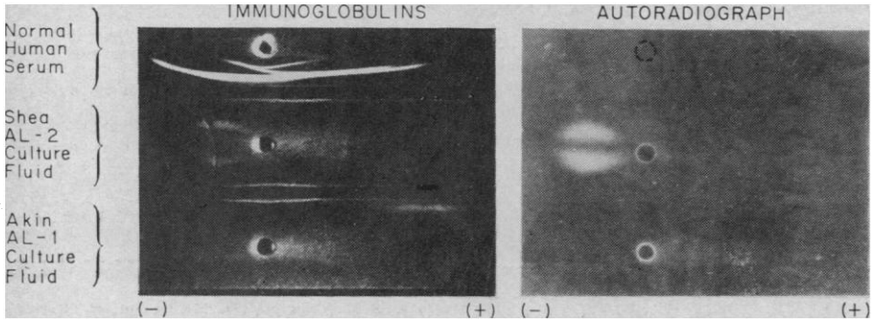

Fig. 1. Immunoelectrophoretic tests of human lymphoma (Burkitt) culture fluids. Culture fluids were collected after cell growth for 48 hours in a medium containing C^{14}-amino acids and were concentrated. After electrophoretic separation of the samples (including normal human serum in the top well for reference purposes) polyvalent antiserum reacting with all human immunoglobulins was added to the troughs. Photograph (left) of the precipitin lines was taken after 48 hours. The agar plate (right) was then washed and dried and exposed to Kodak Royal Pan film for 2 weeks.

munoglobulin synthesis by labeled amino acid incorporation was as follows. Cells (10^7) were collected, washed twice in medium 320 (*9*), and incubated with 1.5 ml of medium containing C^{14}-labeled amino acids. (The medium was made with 100 ml of medium 320 that was deficient in lysine and isoleucine, but with 100 μc of C^{14}-lysine and C^{14}-isoleucine, and 500 mg of ovalbumin added.) After a 48-hour incubation period, the cells were chilled to 4°C, homogenized, and centrifuged at 15,000*g* for 10 minutes. The supernatant was dialyzed against 100 volumes of cold 0.014*M* NaCl solution (three changes and mechanical stirring) for 24 hours to remove unincorporated amino acids. The dialyzate was concentrated tenfold by lyophilization and applied to immunoelectrophoresis plates in the usual manner. Antiserum was added and precipitin lines were permitted to develop for 48 hours. Subsequently the plates were dialyzed in saline for 48 hours and dried overnight, and Kodak Royal Pan film was applied. The film was exposed for 14 days and developed. Incorporation of C^{14}-amino acid into the AL-2 immunoglobulin (Fig. 1) was definite evidence for the synthesis of this protein by the AL-2 cells.

Further characterization of the AL-2 immunoglobulin was carried out by radioimmunoelectrophoresis (Fig. 2). Radiolabeling demonstrates formation of polypeptide γ-chains (heavy) and polypeptide κ-chains (light) by the AL-2 lymphoma line. These findings indicate that this cell line synthesizes IgG molecules of Type K. The IgG product of this cell line is designated IgG-AL2.

The approximate size of IgG-AL2 was estimated by gel filtration. The AL-2–concentrated growth media was fractioned on a column of Sephadex G-200. The IgG-AL2 was eluted in the same region as bovine γ-globulin, after the serum macroglobulins and before bovine serum albumin and in the same volume of distribution as the IgG of normal human serum tested in a subsequent chromatogram on the same column. These findings, although an indirect estimate of the molecular size and shape (*10*), provide good evidence that the IgG-AL2 molecules are similar in size to normal human IgG.

Biosynthetic studies, similar to those shown in Fig. 2, demonstrated that IgG-Type L (λ light chain) molecules (IgG-EB2) are synthesized in a line of EB-2 Burkitt lymphoma cells. Also, IgM molecules (IgM-Og) are synthesized in the Ogun line of Burkitt lymphoma cells.

The AL-2 culture line has been maintained in culture for 10 months. Repeated biosynthetic tests have shown that this line has been producing the same immunoglobulin for the past 4 months. The EB-2 line has been in continuous culture for 2 years and the Ogun line for more than a year. Thus, these cell lines appear to have a well established capacity to produce specific immunoglobulin.

The proteins synthesized in the lymphoma cultures resemble myeloma protein synthesized in plasma-cell tumors. Both myeloma proteins and the immunoglobulins from lymphoma cultures appear relatively homogeneous on zone electrophoresis. Both categories of immunoglobulins are limited to one class each of heavy and light polypeptide chains, that is, are composed of γ or μ (and so forth) heavy chains and κ or λ light chains.

The relative homogeneity of myeloma proteins is generally interpreted as an indication that the proteins are produced by a single clone of malignant cells. The similar relative homogeneity of immunoglobulins from lymphoma cell cultures would be consistent with a clonal origin.

Burkitt lymphoma cells are generally described as lymphoid cells. Histologic analyses have not previously emphasized any relationship to plasma cells. On electron-microscopic examination, however, ribosomal particles may be seen and endoplasmic reticulum detected (*3, 11*). If the Burkitt cells are accepted as lymphoid, then our results support the view that lymphoid cells can produce immunoglobulins.

Lymphoma cells employed in our study were isolated from patients with malignant disease, and particles resembling herpes virus have been demonstrated in them by electron microscopy (*2, 3, 6, 11*). The relation of these viral particles to the patients' malignancies

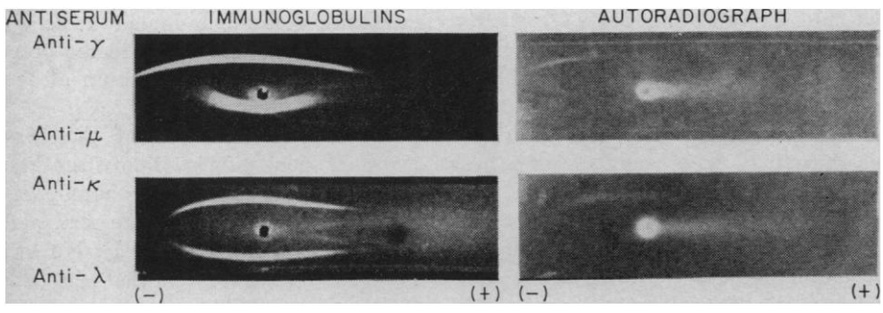

Fig. 2. Radioimmunoelectrophoresis of AL-2 lymphoma cultures. Concentrated culture fluid obtained after 48 hours incubation in medium containing C^{14}-amino acids was mixed with normal human serum and separated by immunoelectrophoresis. Specific antiserums were used to detect polypeptide γ-, μ-, κ-, and λ-chains. Autoradiographic procedures were used to detect the polypeptide γ- and κ-chains characteristically synthesized in the AL-2 culture.

and their infectivity and pathogenicity for man are unknown. Because of the uncertainties about these particles, caution is warranted when working with lymphoma cell cultures.

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Polonium-210 Content of Human Tissues in Relation to Dietary Habit

Abstract. *Concentrations of polonium-210, a natural fallout nuclide, in human placentas collected in northern Canada ranged up to 27.8 picocuries per 100 grams, or 80 times the average United Kingdom value. High levels are related to the inclusion of reindeer or caribou meat in the diet, and a correlation exists between the concentrations of polonium-210 and cesium-137 in the placentas.*

Attempts to follow up the suggestion (*1*) that there may be relatively high levels of Po^{210} in tissues of people dependent for food on meat of animals, such as reindeer and caribou, that graze on lichens have hitherto been prevented by difficulties in obtaining suitable samples of tissue. I now report on measurements made on a series of human placentas obtained from residents of northern Canada, as compared with a series from London, United Kingdom. The Canadian series was obtained from 32 subjects resident in the following three general areas of the Yukon and Northwest Territories: Area No. 1, the town of Yellowknife; Area No. 2, rural inland districts in the Mackenzie River and Southern Yukon region; and Area No. 3, the northwest shores of Hudson Bay. The subjects can also be classified in the following three groups on the basis of dietary habit: (i) reindeer or caribou meat consumed several times a week, (ii) either reindeer or caribou meat consumed about once a week or a diet described generally as "high protein," and (iii) diet not described as "high protein" or not including reindeer or caribou meat (that is, "normal"). No dietary record was obtained for the English series of ten samples and, because the samples were collected unselectively and in the sequence in which they were delivered in a general hospital, they can be presumed to be representative of the area where they were obtained.

Determinations of the concentration of Po^{210} in these samples were carried out by a method previously described (*1*), and results are given in Table 1. Values for the Canadian "normal diet" samples are comparable to those for the English series, while the value for the caribou-reindeer eaters are generally higher than the "normal" mean value by factors of up to 80. This is about the same range of variation found in earlier measurements on samples of Eskimo bones (where, however, no information on diet was available) (*1*). The results (Table 1) also indicate a dependence of Po^{210} concentration on residence locality, as such, among subjects within a given diet classification. However, this may simply reflect the inadequacy of the information available to us concerning detailed dietary habits of subjects in the areas concerned. The values of the activity ratio of Pb^{210}/Po^{210} (shown in parentheses) that have been measured for some of the samples show, in every case, that Po^{210} is in excess of equilibrium with Pb^{210}.

Estimation of the Po^{210} concentrations in other body organs of the Canadian residents can be made by comparison of the English placenta series with a previous set of measurements of Po^{210} in autopsy samples from accident cases occurring in the same area as that from which the placenta samples were drawn (*1*). This comparison is made in Table 2. Unfortunately bone was not included in this series of measurements; a value for bone has been derived from the concentration ratio, bone/liver, found in another set of measurements of autopsy material, which was carried out on tissue from hospitalized subjects with terminal illness (*2*).

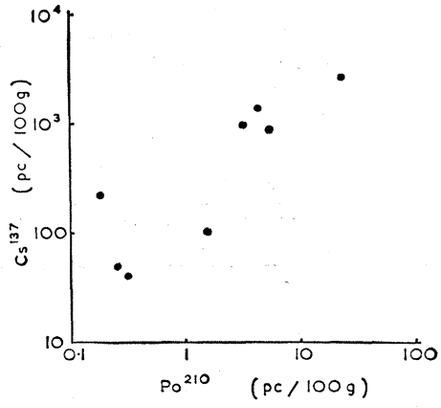

Fig. 1. Relationship between Po^{210} and Cs^{137} concentrations measured in human placentas.

The ratios given in the third column of Table 2 should provide a means for estimating the Po^{210} concentration in the corresponding tissues of the subjects of Table 1, and in particular the "meat eaters." In this connection it is useful to bear in mind that a Po^{210} concentration of 1 pc/100 g, uniformly distributed, gives rise to a tissue dose rate of about 1 mrad/yr.

Table 1. Polonium-210 concentration, in picocuries per 100 g (wet wt.) in human placenta. Activity ratios, Pb^{210}/Po^{210}, are shown in parentheses.

Yellowknife, N.W.T.	Inland, rural	Hudson Bay coast	London, U.K.
Much reindeer and caribou			
2.41 (0.25)	1.54	4.3	
	5.28 (0.16)	5.4	
		9.3	
		12.2	
		14.1	
		22.8	
2.41	3.41	11.4 ± 6.2	
Some reindeer and caribou, or "high protein"			
0.35	0.09	3.2	
	1.07 (0.64)	5.4	
	1.48 (0.75)	9.6	
	3.09 (0.27)	10.8	
		11.5	
		12.2	
		27.8	
0.35	1.44	11.5 ± 7.3	
"Normal" (no reindeer or caribou)			
0.08			0.14
0.18			0.23
0.19			0.26
0.24			0.27
0.30			0.28
0.31			0.29
0.36			0.37
0.40			0.39
0.45			0.50
0.58			0.52
0.92			
0.36 ± 0.22			0.33 ± 0.11