and location of other bacterial cell culture contaminants which may be detected by this technique, and their capacity to grow in simple bacteriological media, distinguished them easily from mycoplasma.

Summary. Direct microscopic observation of pleuropneumonia-like organisms (PPLO) in cell cultures is easily accomplished following hypotonic treatment, air-drying and staining with orcein. A rapid technique using FL human amnion cells, inoculated with supernatant from suspected cultures, is described. The demonstration of PPLO contamination of 30 cell lines by this rapid method was in complete agreement with results of PPLO-agar techniques.

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Subclasses of Human IgG Molecules Differing in Heterologous Skin Sensitizing Properties. (29732)

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Subclasses of normal IgG (γ_2-immunoglobulin) molecules have recently been detected (1-6). These subclasses are based on differences in antigenic determinants, and G-myeloma proteins related to each subclass have been identified.

IgG molecules are composed of 2 different types of polypeptide chains, the heavy (γ) and light (κ or λ) chains. The distinctive characteristics of molecules in the IgG subclasses designated γ_{2a}, γ_{2b} and γ_{2c}-globulins* are due to antigenic differences among the heavy chains(3,6). These differences have been further localized to the Fc (fast migrating) fragment, obtained after papain digestion(3,6).

Studies in other species have shown that the Fc fragment of IgG molecules is responsible for several biologic properties, including their ability to sensitize skin of heterologous species for the passive cutaneous anaphylaxis (PCA) reaction(8-10). Since the γ_{2a}, γ_{2b} and γ_{2c} subclasses of IgG differ in Fc fragment antigenic characteristics, possible differences in the skin-sensitizing properties of these molecules were investigated. The γ_{2a}, γ_{2b} and γ_{2c}-globulins have not yet been individually purified from normal serum. However, G-myeloma proteins representing each of the subclasses were available. The ability of these myeloma proteins to sensitize heterologous (guinea pig) skin was investigated with the technic of reverse passive cutaneous anaphylaxis (RPCA).

Materials and methods. Normal human

* This terminology is consistent with the earliest designations of IgG subclasses(1,3). When information is available concerning the interrelationships of these subclasses, more appropriate designations, conforming to the recommendations of the Committee on Human Immunoglobulin Nomenclature(7) can be made.

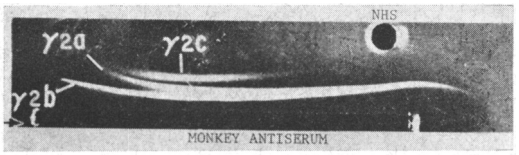

FIG. 1. Immunoelectrophoresis of normal human serum (NHS). The γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins are revealed by monkey antiserum to human IgG (γ_2-immunoglobulin).

IgG (Hyland Laboratories, 442 R 11) was further purified by anion exchange chromatography on diethylaminoethyl (DEAE) cellulose(11). G-myeloma proteins were isolated from serum or plasma by DEAE-cellulose chromatography, or by a combination of block (zone) electrophoresis and chromatography(11).

Isolated myeloma proteins were identified as γ_{2a}, γ_{2b} and γ_{2c}-globulins by Ouchterlony analysis using specific monkey antiserums (6). The corresponding components of normal human serum are illustrated in Fig. 1.

Reverse passive cutaneous anaphylaxis tests were performed as described by Ovary (12). Purified human IgG and individual G-myeloma proteins (0.1 ml) were injected intradermally (ID) into 300 g albino guinea pigs of the Hartley strain. Three concentrations (100, 10 and 1 μg/ml) of each myeloma preparation were tested in 4 different guinea pigs. In addition, every animal received an ID injection of pooled human IgG at a concentration of 100 μg/ml to serve as a positive control. Four hours after the ID injections, 1 ml of 1/10 dilution of rabbit anti-IgG antiserum, made 0.5% in Evans blue dye, was injected intravenously. The rabbit antiserum had been absorbed with an equal volume of guinea pig kidney homogenate, and was shown to react with γ_{2a}, γ_{2b} and γ_{2c}-myeloma proteins by precipitation in agar. Skin sites were examined 30 minutes after the intravenous injection, and diameters of the blue spots were measured. To compensate for asymmetry of the spots, the longest diameter of a spot was averaged with the diameter perpendicular to it. The resulting average diameters are reported.

Results. Myeloma proteins of subclasses γ_{2b} and γ_{2c} fixed to guinea pig skin and yielded positive RPCA reactions similar to those caused by normal human IgG (Tables I, II). γ_{2a}-myeloma proteins did not give positive RPCA tests. Results of studies with an individual γ_{2a}, γ_{2b} and γ_{2c}-myeloma protein injected into 4 guinea pigs are in Table I.

Investigations of 15 G-myeloma proteins are summarized in Table II. Each of five γ_{2a}-myeloma proteins failed to sensitize guinea pig skin for the RPCA reaction. In contrast, each of the six γ_{2b} and four γ_{2c}-myeloma proteins gave positive RPCA tests.

Discussion. Human γ_{2a}, γ_{2b} and γ_{2c}-myeloma proteins differ in their ability to sensitize guinea pig skin for the RPCA reaction. Previous studies of these IgG subclasses indicate that the molecules differ in antigenic characteristics of the heavy polypeptide chain and, more specifically, of the Fc (fast migrating) fragment obtained after papain digestion(3,6). The Fc fragment of human IgG is considered to represent at least a por-

TABLE I. RPCA Tests of γ_{2a}-, γ_{2b}- and γ_{2c}-Myeloma Proteins in the Skin of 4 Guinea Pigs.

IgG component	Concentration (μg/ml)	Diameter of reaction (mm) GP1	GP2	GP3	GP4	Avg diameter (mm)
Pooled normal human IgG	100	23	16	20	28	21.8
γ_{2a}-Myeloma protein	100	0	0	0	0	0
	10	0	0	0	0	0
	1	0	0	0	0	0
γ_{2b}-Myeloma protein	100	25	18	21	25	22.3
	10	17	10	15	15	14.3
	1	5	tr*	5	tr	2.5
γ_{2c}-Myeloma protein	100	18	13	25	23	19.8
	10	14	9	15	18	14.0
	1	tr	0	10	tr	2.5

* tr = trace reaction.

TABLE II. Summary of RPCA Tests of 15 Human G-Myeloma Proteins in Guinea Pig Skin.

IgG component	No. of different IgG preparations tested	Concentrations (μg/ml)	Avg diameter
Pooled normal human IgG	1	100	18.4*
		10	11
		1	0
γ_{2a}-Myeloma proteins	5	100	0
		10	0
		1	0
γ_{2b}-Myeloma proteins	6	100	18.8
		10	13.1
		1	2.2
γ_{2c}-Myeloma proteins	4	100	18.4
		10	13.5
		1	1.5

* Avg value from three different tests using 20 guinea pigs.

tion of the molecule responsible for skin-sensitizing properties(13). This hypothesis has not been directly tested, since all fragments derived from enzymatic digestion of normal human IgG lack skin-sensitizing activity(13). The data presented here are consistent with this hypothesis, since the only detectable differences between skin-sensitizing γ_{2b} and γ_{2c}-molecules, and non-skin sensitizing γ_{2a}-molecules are the antigenic characteristics of their Fc fragments. The nature of the relationship between antigenic characteristics and skin-sensitizing properties of the heavy polypeptide chain are unknown.

Light polypeptide chains of G-myeloma proteins may be of antigenic type κ (I) or λ (II)(14-16). Myeloma proteins of both light chain types were tested by RPCA (Table III). There is no correlation between type of light chain and ability of the molecule to sensitize guinea pig skin. This is in accord with the evidence that skin sensitization is a property of the heavy polypeptide chain(8-10).

At least two reactions are involved in the development of a positive RPCA test. First, the protein injected into the skin must bind to some dermal structure. If this does not occur, the injected protein diffuses out of the test site during the 4-hour latent period between ID injection and intravenous challenge and is not present to combine with specific antibodies. Second, combination of the challenge (antiserum) antibodies with skin-fixed antigen must initiate reactions which cause the increased permeability required for a positive RPCA test. The negative RPCA tests obtained with γ_{2a}-myeloma proteins could have occurred because γ_{2a}-molecules failed to bind to guinea pig skin, or because combination of γ_{2a}-molecules with antibody failed to cause increased vascular permeability. Studies are in progress to investigate the basis for the negative RPCA tests of γ_{2a}-molecules.

The concentrations of γ_{2a}, γ_{2b} and γ_{2c}-globulins in pooled normal human IgG were determined by antibody in agar diffusion plate technics(17). Approximately 30% of the IgG molecules are of the γ_{2a} subclass. If normal human IgG γ_{2a}-molecules are functionally similar to γ_{2a}-myeloma molecules and do not fix to guinea pig skin, the maximum skin sensitizing dose of IgG used as a positive control was 70, 7 and 0.7 μg/ml, rather than the total protein concentrations of 100, 10 and 1 μg/ml given above. RPCA test findings with the normal IgG preparation are quantitatively consistent with the results obtained with individual myeloma proteins, *i.e.*, a smaller average diameter was obtained with 10 μg/ml of normal IgG than with the same concentration of γ_{2b} of γ_{2c}-myeloma proteins (Table II).

Studies of the immunoglobulins of guinea pigs(18) and mice(19-21) have also revealed 2 populations of 7S immunoglobulin molecules that differ in their ability to sensitize the skin of heterologous species for PCA. The slower migrating 7S γ-globulins (7S γ_2-globulins) give positive PCA reactions in heterologous species. The faster migrating molecules (7S γ_1-globulins) of both guinea pig and mouse fail to sensitize heterologous skin. Parallel differences are noted in the present

TABLE III. Distribution of Type κ (I) and Type λ (II) Light Chains Among 15 γ_{2a}-, γ_{2b}- and γ_{2c}-Myeloma Proteins Used for RPCA Tests.

G-myeloma protein heavy chain subclass	Light chain types	
	κ (I)	λ (II)
γ_{2a}	3	2
γ_{2b}	6	0
γ_{2c}	2	2

experiments in which the faster migrating group of human γ_{2a}-globulin molecules (Fig. 1) fail to sensitize heterologous skin. Further investigation will be required to determine if the human, mouse and guinea pig subclasses are comparable.

Summary. The γ_{2a}, γ_{2b}, and γ_{2c}-globulin subclasses of human serum IgG were tested for their ability to sensitize heterologous (guinea pig) skin. In studies using 15 G-myeloma proteins, positive RPCA tests were obtained with γ_{2b} and γ_{2c}-myeloma proteins. In contrast, γ_{2a}-myeloma proteins gave negative RPCA reactions. These results indicate that the heavy polypeptide chains of γ_{2a}-globulin molecules differ from the heavy chains of γ_{2b} and γ_{2c}-globulin molecules in skin sensitizing as well as in antigenic properties.

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Relationship Between Lymphatic and Blood Flow in Various Structures in the Abdominal Cavity.* (29733)

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It is generally taught that lymph flows from the tissues of the lower extremities and the abdominal organs into the cisterna chyli and then through the thoracic duct to empty into the venous system at the root of the neck. Based on this concept the fraction of various substances absorbed from the alimentary canal into the lymphatic system has been calculated from concentrations and flows in the thoracic duct(1,2,3). The persuasive morphological evidence(4,5) that lymph might empty into the venous system by alternative lymphatico-venous anastomoses has been disregarded(6). We have adduced pertinent information bearing on this point from studies of tissue clearance of diffusible and non-diffusible molecules.

Hyman and Freedman(7) found that the rates of removal of a small dye molecule (Water Blue) and a protein-bound dye (T-1824 albumin) from microinjection sites in frog mesentery differed, but the ratio of clearance of diffusible to clearance of colloidal

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