to be reached at 218 days. Other interpretations are possible with present knowledge, and additional studies of the influence of duration and level of drug on bacterial killing are being carried out.

The duration of the activity of DADDS in mice and monkeys was studied by Thompson, Olszewski, and Waitz(4), who gave a single injection of drug and challenged with malaria parasites at intervals thereafter. In mice doses of 100 to 400 mg/kg in benzyl benzoate and castor oil suppressed *Plasmodium berghei* for 6 to 14 weeks; 50 mg had little activity at one week. In monkeys doses of 50 mg/kg in the same vehicle prevented infection of *P. cynomologi* for 9 to 38 weeks. In man DADDS has suppressed falciparum malaria infections for about 60 days(10).

In leprosy effective repository drugs might have special usefulness, both in treatment and chemoprophylaxis, because of the very limited medical facilities in many of the important endemic areas.

Summary. 1. Seven discrete repository sulfones were tested against *M. leprae* in mice, and all were found completely suppressive when injected at 2-month intervals. DDS was inactive when given at 2-month intervals, partially suppressive at one month, and completely suppressive at half-month intervals. 2. The repository sulfone 4,4′-diacetyldiaminodiphenylsulfone was titrated in mice against *M. leprae*. The least dose giving nearly complete suppression was 6 mg/kg/2 months. Cycloguanil pamoate, a repository triazine antimalarial, was inactive in a dose of 400 mg/kg/2 months. 3. Further tests of the sensitivity of *M. leprae* to DDS were carried out by feeding the drug in the diet. Two strains were completely inhibited by 0.0001%; the same two and two other strains were insensitive to 0.00001%.

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Sites of Production of Primate Serum Proteins Associated with the Complement System.* (31758)

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Various complement components have been characterized as individual serum proteins which are distinguished by their physiochemical and immunological properties. Four of the components can be identified in immunoelectrophoretic patterns. Among these are C′3 as β_{1C}(1,2), C′4 as β_{1E}(3), C′5 as β_{1F}(4), and C′1q as an 11S globulin of γ-mobility(5,6,7). The technique employing autoradiography of immunoelectrophoretic

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(IE) patterns for the demonstration of incorporation of C^{14}-amino acids into serum proteins by tissues *in vitro* can, therefore, be used for the study of the sites of synthesis of proteins associated with the complement system(8,9,10). In previous studies concerning only β_{1C} and β_{1E}, it has been found that various primate tissues, such as liver, spleen and other tissues of the reticuloendothelial system, as well as a few epithelial organs, incorporate C^{14}-amino acids into these proteins(10).

Such a wide distribution over various tissues is not observed for albumin and several other proteins which are only formed in the liver(11,12), and for the immune globulins which are formed primarily in lymphoid tissues(8,9,10). In an attempt to identify a specific cell type capable of β_{1C} production, peritoneal exudate cells of rodents were examined. It was found that peritoneal macrophages are very active in the synthesis of β_{1C}(13). It is important to establish whether other complement components are also formed by many different tissues and by peritoneal macrophages. The present studies concern, therefore, the synthesis of the 11S globulin (C′ 1q) *in vitro*.

Materials and methods. Rhesus monkey tissues were obtained immediately after exsanguination of the animal. Peripheral blood leucocytes were isolated by dextran sedimentation. Peritoneal macrophages were collected by rinsing the peritoneal cavity with sterile heparinized Hanks' balanced salt solution. Lung macrophages were obtained by the methed of Myrvik *et al*(14). Human tissues were from biopsy material, and thoracic duct cells were procured by thoracic duct cannulation. Human peritoneal macrophages were collected from ascites fluid. In some cases the lung and peritoneal macrophages were further isolated from other cell types by allowing them to attach to a glass surface for one hour at 37°C before rinsing away non-adhering cells and incubating with the C^{14}-amino acid containing medium.

Tissues or cells were cultured for 24 hours in a modified Eagle's medium containing 0.5% ovalbumin, and 1 μc each of C^{14}-lysine (600-1500 μc/mg) and C^{14}-isoleucine (675-2000 μc/mg) per ml. The resultant tissue culture fluids were dialyzed, concentrated by lyophilization, and analyzed by means of autoradiography of IE patterns(8,9,11). Since the culture fluids contained only minute amounts of the individual labeled serum proteins, human sera, human or monkey euglobulin, or human β_{1E} were used as carriers. Human and monkey euglobulin preparations were obtained as previously described(7). IE analysis for the 11S globulin was performed according to the method of Morse and Christian (7). The IE patterns were developed using specific rabbit antisera to human β_{1C}, β_{1E} (Lloyd Brothers, Inc., Cincinnati, Ohio) or the 11S globulin(7), as well as by anti-whole human serum. Heating for 30 minutes at 56°C abolished the precipitin arc for 11S globulin. The properties of monkey 11S globulin were found to be quite similar to those of its human homologue.

TABLE I. Labeling of 11S Globulin by Various Monkey Tissues *in vitro*.

Tissue	No. of cultures	Degree of labeling* +	w+	—
Liver—Stimulated†	4	2	2	
Normal	3		1	2
Fetal	2	1	1	
Spleen—Adult	5	3		2
Fetal	2	2		
Bone marrow	5	1	1	3
Mesenteric lymph node	3		1	2
Lung	2	1		1
Kidney	3		1	2
Fimbriae	4		1	3
Thyroid	3			3
Salivary gland	4			4
Endometrium	2			2

* The intensities of the autoradiographic images were graded as +, w+, or negative.

† Intravenous injection of endotoxin or living pneumococci, as well as partial hepatectomy, were shown to be effective in stimulating serum protein synthesis by the liver(10,12).

Results. A summary of the results for 11S globulin production by monkey tissues is given in Table I. It can be seen that cultures of liver, spleen and other reticuloendothelial tissues such as bone marrow, lung, and mesenteric lymph node, as well as a few cultures of epithelial organs (kidney and fimbriae ovarium) labeled the 11S protein. This distribution is fairly similar to the one observed for β_{1C} and β_{1E} production, except that label-

TABLE II. Labeling of Complement Proteins by Various Primate Cell Types *in vitro*.

Cell type	No. cells cultured	β_{1C} (C′3)	β_{1E} (C′4)	11S (C′1q)
Monkey				
Perit. macr.	.6 $\times 10^7$‡	1/1*	1/1	1/1
Lung macr.	.3-1.8 $\times 10^7$‡	3/3	0/3	2/2
Periph. leuk.	.3 $\times 10^7$	0/3	0/3	0/3
Human				
Perit. macr.	1 -4 $\times 10^7$‡	3/3	2/3	2/3
Thor. duct ly.	.9-8 $\times 10^7$	2/9	2/9	2/3
Periph. leuk.	1 $\times 10^7$	0/3	0/3	N.T.†

* Fractions indicate number of cultures labeling this protein out of total number tested.
† N.T. = Not tested.
‡ No. of cells present prior to macrophage isolation.

ing by epithelial organs was noted less frequently for the 11S globulin.

Human tissue rarely labeled the 11S globulin. An occasional spleen (1/2) and liver (1/4) showed labeling, but the majority of the cultures did not. Included in the negative group were 2 thyroid, 2 lung, 2 mammary gland and 2 bone marrow cultures.

It was found that various human tissues synthesize β_{1C} and β_{1E} and that there is a close similarity in the labeling patterns produced by monkey and human tissues. With the method used in the present studies 11S production was demonstrable more frequently with monkey than with human tissues. This may be the result of a difference in sensitivity due to the antigen-antibody systems used as carriers, or else reflect a lower level of synthesis for the 11S protein in the human than in the monkey.

The previously initiated search(13,15) for a specific cell type involved in the synthesis of complement components was extended. Cultures of peritoneal exudate cells and macrophages isolated from such cell suspensions, as well as from lung, were included. In addition, a few more cultures of peripheral blood leucocytes and of thoracic duct cells were examined. The results for synthesis of β_{1C}, β_{1E}, and 11S globulin by these different isolated cell types of both human and monkey origin are summarized in Table II. The only cell types which consistently labeled all 3 proteins studied were peritoneal and lung macrophages. Human and monkey peritoneal macrophages specifically isolated by adherence to a glass surface still labeled β_{1C}, β_{1E} (Fig. 1) and 11S globulin (Fig. 2). The labeling of immune globulins which was often observed in cultures of unfractionated primate peritoneal macrophages was much less prominent after such isolation procedures (Fig. 1). Monkey lung macrophages labeled β_{1C} and the 11S globulin.

Although it was previously found that thoracic duct cells and peripheral blood leucocytes did not incorporate C^{14}-amino acids into β_{1C} and β_{1E}, the present investigation showed that 2 out of a total of 9 thoracic duct cultures contained cells capable of β_{1C} and β_{1E} production. These two cultures also labeled the 11S globulin. It should be noted that one of these cultures was prepared from thoracic duct cells of a patient with rheumatoid arthritis. Unfortunately there remained insufficient amounts of many of the early thoracic duct cell culture fluids(10) to allow testing for the presence of labeled 11S globulin.

Discussion. These results demonstrate that peritoneal macrophages incorporate C^{14}-amino acids into various serum proteins associated with complement activity. No such activity could be demonstrated in peripheral blood leucocytes, and in most of the thoracic duct lymphocyte cultures. Studies on β_{1C} synthesis by different cell types in the rat have given similar results(13,15).

Many of the other tissues found to produce β_{1C}, β_{1E}(10), or 11S globulin, may contain a sufficient number of macrophages to account for this synthesizing activity. However, certain discrepancies, such as the observation that most of the kidney and thyroid cultures contained labeled β_{1E}, are not readily explained on this basis. It should also be noted that the normal liver produces some β_{1C}, and that the liver, showing enhanced serum protein synthesis after stimulation by various procedures, may show production of β_{1C}, β_{1E}(10) and 11S globulin. It has not been determined which cell type in the normal and in the stimulated liver synthesizes these proteins. Attempts to separate Kupffer and parenchymal cells in a condition allowing for continued serum protein synthesis have failed. Studies with specific fluorescein-labeled anti-β_{1C} have

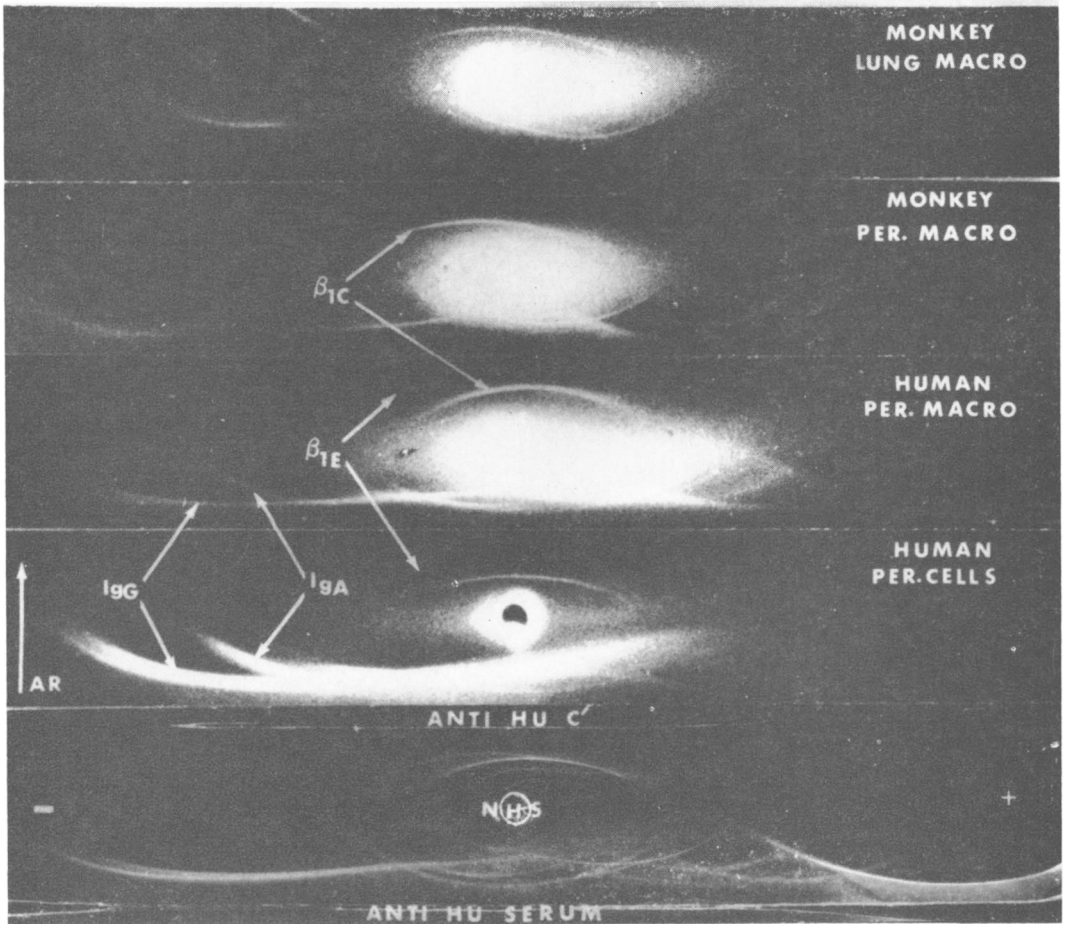

FIG. 1. Autoradiographs (AR) of immunoelectrophoretic patterns prepared with culture fluids from primate lung and peritoneal (PER.) macrophages (MACRO.). Normal human serum (NHS) was used as the carrier and the patterns were developed with rabbit antisera to human serum and to human complement. Note that macrophages isolated from human peritoneal exudates showed equally intensive labeling of β_{1C} and β_{1E} as did the total mixture of peritoneal exudate cells. Labeling of IgA and IgG, on the contrary, was much reduced. The protein of γ-mobility which is labeled by the monkey macrophages represents γ-trace(22).

also been unsuccessful, probably due to lack of sensitivity of this procedure. The technique used in the present studies for the demonstration of C^{14}-amino acid incorporation is quite sensitive, detecting quantities as small as approximately 0.01 μg synthesis per culture tube(16).

Although this method of demonstrating incorporation of C^{14}-amino acids is both sensitive and specific, certain limitations should be kept in mind. At most it can demonstrate synthesis of serum proteins with typical immunoelectrophoretic properties. It shows neither net synthesis nor biological activity of a protein, and the possibility of artifacts created by the complexing of serum proteins with other labeled tissue products cannot be completely ruled out. Other studies have shown that a few serum proteins may exhibit such complexing(17,18). In the case of complement components protein-protein interaction may occur between 11S protein and γ-globulin(6) and within the complement system for the 3 subcomponents of C′1(19) and for C′5, C′6 and C′7(20). This should be taken into account particularly where the labeling of 11S globulin is concerned. It should be realized that simple adsorption of C^{14}-

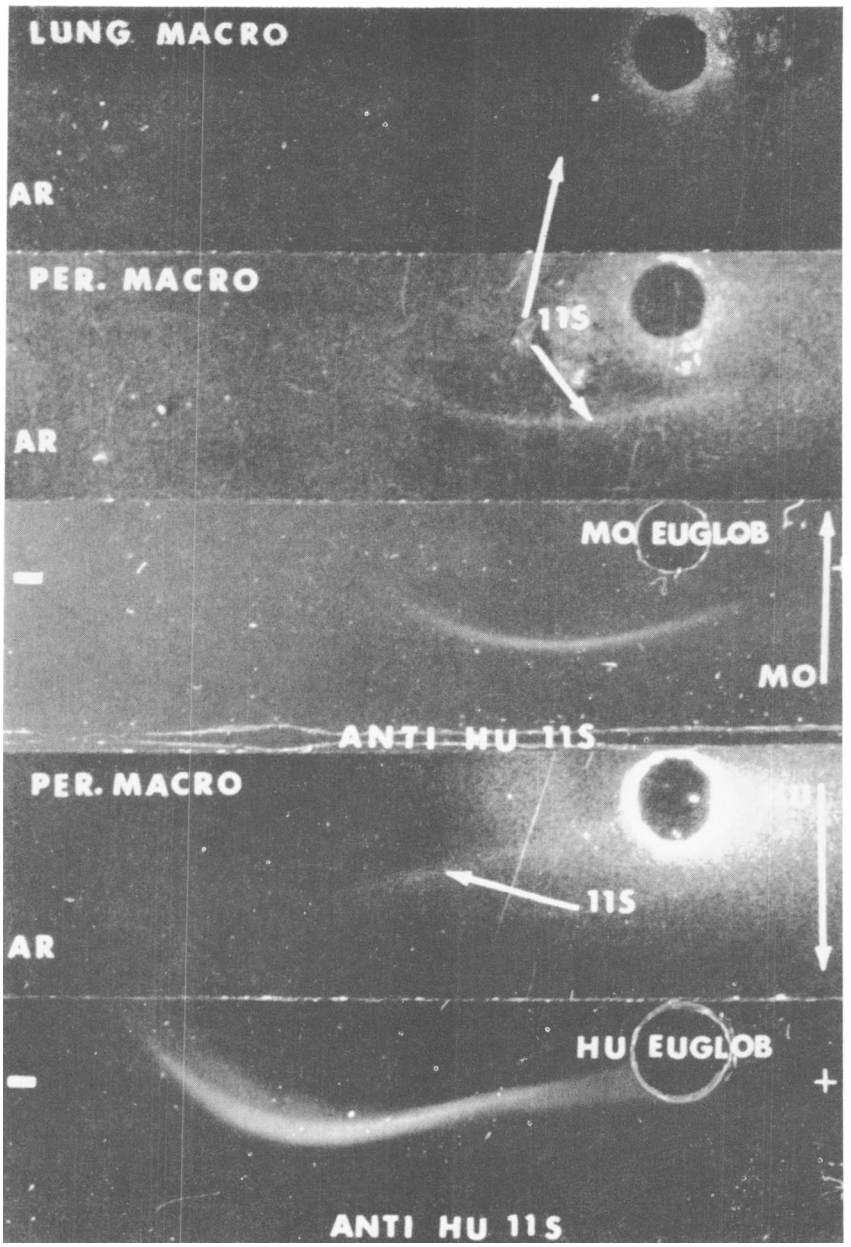

FIG. 2. Autoradiographs (AR) of immunoelectrophoretic patterns prepared with culture fluids from primate lung and peritoneal (PER.) macrophages (MACRO.). Monkey (Mo) or human (Hu) euglobulin preparations were used as carriers, and the 11S precipitin lines developed by rabbit anti-human 11S globulin.

amino acids onto serum proteins has been ruled out as a cause of the labeling observed with this technique(8,16).

Even if macrophages are not the only cell type active in the synthesis of complement factors, the possibility that they may constitute an important site of synthesis for such proteins is of interest. The fact that macrophages may produce complement factors needs to be taken into consideration when evaluating the role of complement in the adherence to and ingestion by macrophages of particu-

late substances(21).

Another question which remains unanswered is whether all these proteins are formed by the same or by different cells. It is of interest in this regard that a human diploid fibroblastic cell strain has been recently found to incorporate C^{14}-amino acids into β_{1C}, β_{1E} and the 11S globulin(15).

Summary. Various human and monkey tissues, including liver, spleen, bone marrow, and lung were found to incorporate C^{14}-amino acids into 11S globulin (C′1q) *in vitro*. A comparison with previous studies on β_{1C} (C′3) and β_{1E} (C′4) production revealed a striking similarity in the sites of synthesis of these 3 proteins. Primate macrophages isolated from peritoneal exudates or lungs were particularly active in the synthesis of β_{1C}, β_{1E}, and 11S globulin. It was discussed that macrophages of various species appear to constitute a major site of production of several complement components.

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