SITES OF FORMATION OF IMMUNE GLOBULINS AND OF A COMPONENT OF C'_3

II. Production of Immunoelectrophoretically Identified Serum Proteins by Human and Monkey Tissues In Vitro

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Studies on the sites of protein formation have demonstrated synthesis of serum albumin, fibrinogen, and α- and β-globulins in the rat liver (1), of ovalbumin by chicken liver slices *in vitro* (2), and of gamma globulin and antibody by various rabbit organs containing reticuloendothelial tissue, such as spleen, lymph nodes, lung, and bone marrow (3, 4). Perhaps, it can be assumed that serum protein production proceeds at similar sites in the human, but there is little direct evidence bearing on this problem. Most of the evidence that does exist concerns serum proteins serologically related to gamma globulin, β_{2A}-globulin, and β_{2M}-globulin (5–7). Fluorescein-labeled antibodies have proved useful in showing the presence of such proteins in normal and abnormal plasma cells (8–11). Transplantation of a lymph node from a normal donor to a patient with hypogammaglobulinemia was shown to be followed by a rise in gamma globulin in the patient's serum (12). Production of myeloma and Bence-Jones proteins has also been demonstrated in cultures of bone marrow tissue from patients with multiple myeloma (13). Levene *et al.* (14) found that C^{14}-lysine was incorporated into 19 S gamma globulin by lymph nodes, *in vitro*, from patients with rheumatoid arthritis. This was not demonstrable in cultures of nodes from normal subjects.

In the accompanying paper a method has been described (15) in which immunoelectrophoresis and autoradiography are utilized to detect radioactively labeled serum proteins synthesized by tisues *in vitro* in the presence of C^{14}–amino acids. Much more is known about the distribution and characterization, upon immunoelectrophoresis, of human than of animal serum proteins. It seemed appropriate, therefore, to apply this technique to the study of serum protein production by human tissues, *in vitro*. In addition, since many of the serum proteins of certain species of monkey are immunochemically closely re-

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lated to human serum proteins, and give similar lines upon immunoelectrophoresis (16), tissues from an appropriate monkey were also used.

Materials and Methods

Tissues.—Thirteen lymph nodes were obtained from patients with various diseases, in the course of elective operative procedures performed at Bellevue Hospital, New York. Nodes from the cervical, mesenteric, and inguinal regions were included. Insufficient amounts of tissue were obtained for histologic study in most cases, but the nodes were grossly normal in appearance.

Two specimens of grossly normal ileum were obtained from patients with carcinoma of the colon. The tissues were taken from resected portions of intestine, at points distant from the tumor sites. The only other specimen of intestinal tissue used for tissue culture was a portion of normal appendix.

Three normal human bone marrows were obtained by aspiration from the sternum. An additional specimen of bone marrow from a rib resected during thoracotomy was also used.

A squirrel monkey (*Saimiri sciurea*) was killed by exsanguination, and tissue cultures were made of spleen, bone marrow, liver, lung, ileum, colon, thymus, adrenal, lymph node, and kidney.

Medium.—The culture medium consisted of Hanks' balanced salt solution, 0.5 per cent ovalbumin, mixtures of vitamins (17), antibiotics, and of amino acids (18) either without lysine or without isoleucine. Uniformly labeled C^{14}-L-isoleucine (675 μc/mg, Institut Pasteur, Paris) 2 μc/ml, was added to the medium without isoleucine, and uniformly labeled C^{14}-L-lysine (605 μc/mg, Institut Pasteur) 2 μc/ml, to the medium without lysine.

Cultures.—The tissues were minced and cultured in roller tubes at 37°C for 24 hours. Approximately 50 mg of tissue (wet weight) was incubated with 2 ml of medium. Only medium containing C^{14}-isoleucine was used in the studies with human tissue. Duplicate cultures of the monkey tissues were made, one with the medium containing C^{14}-isoleucine, the second with the medium containing C^{14}-lysine.

After the 24 hour culture period the culture tubes, containing both medium and tissue were frozen and thawed once. The tissue was then removed by centrifugation at 10000 *g*. The supernatant fluid was dialyzed against 0.15 M NaCl for 24 hours, and subsequently concentrated 10 to 20 times by vacuum dialysis. The concentrated fluids (0.1 to 0.2 ml) were stored at −10°C.

Antisera.—Horse anti-whole human serum, lot 128, was obtained from the Institut Pasteur, Paris.

Rabbit anti-whole human serum: Each of two rabbits was injected, subcutaneously and in the foot-pads, with a mixture of 5 ml human serum and 5 ml complete Freund's adjuvant. 4 weeks later they received, at 3 day intervals, 3 intravenous injections of 1 ml human serum. The animals were bled on the 7th and 8th days after the last injection.

Rabbit antihuman complement serum was obtained through the kindness of Dr. P. G. H. Gell, Department of Experimental Pathology, University of Birmingham, Birmingham, England. Its major line of precipitation upon immunoelectrophoresis with normal human serum corresponded to the β_{1C}-protein described by Müller-Eberhard (19).[1]

Immunoelectrophoresis.—Immunoelectrophoresis and subsequent autoradiography were carried out as previously described (15). The antigen reservoir was filled with "carrier" serum, selected to give lines of precipitation with the various antisera. After the carrier serum had been absorbed into the agar, culture fluid was added to the same reservoir. When the immuno-

[1] The authors are grateful to Dr. H. J. Müller-Eberhard for the gift of some rabbit antiserum specific for β_{1C}-globulin, which allowed final identification of this line.

electrophoretic patterns were developed labeled proteins were precipitated by the various antisera together with the homologous proteins in the carrier serum. Normal human serum, and serum from a patient with macroglobulinemia were used as carriers in the studies with human tissues. These sera, and isologous monkey serum were used as carriers in the studies with monkey tissues. Slides in which normal human serum or isologous monkey serum were used were developed with horse anti-whole human serum, with rabbit anti-whole human serum, or with rabbit antiserum against β_{1C}-globulin. Slides in which macroglobulinemic serum was used were developed with the horse antihuman serum.

Identification of Lines.—Albumin, gamma globulin, β_{2A}-globulin, and β_{2M}-globulin were identified by their characteristic bands of precipitation (20). The α_{1A}-globulin (21), owing to its strong antigenicity and relatively constant level in serum, also gave a precipitation arc of characteristic shape and position with most antisera (20).

Transferrin: 1 ml of human serum was incubated for 1 hour at 37°C with 0.5 ml of Fe^{59} citrate[2] containing 1 μc/ml, 9.93 mc/mg Fe^{59}. Immunoelectrophoresis and autoradiography performed in the usual manner showed a single radioactive arc, of characteristic position in the β_1-region. This result was obtained with both antihuman sera.

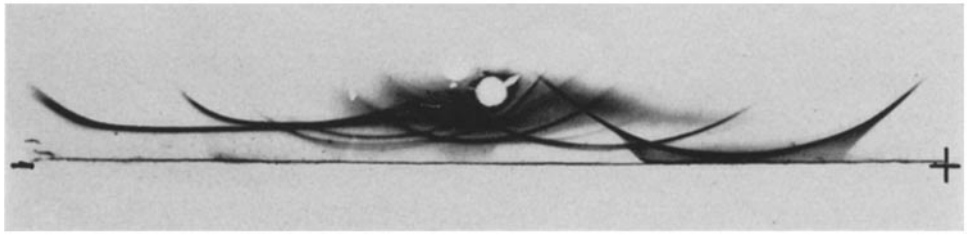

FIG. 1. The immunoelectrophoretic pattern of macroglobulinemic serum, developed with horse anti-whole human serum.

Haptoglobin: A few slides were stained for peroxidase activity (22). Immunoelectrophoretic patterns developed with rabbit anti-whole human serum showed a single blue arc in the α_2-region. Slides developed with horse anti-whole human serum showed, in addition to this arc, a 2nd arc in the β_1-region with less peroxidase activity.

Ceruloplasmin: Staining for ceruloplasmin oxidase activity (22) revealed a single brown arc in the α_2-region.

RESULTS

Immunoelectrophoresis.—The commercially obtained anti-human serum gave 15 arcs of precipitation with normal human serum (Fig. 1). Albumin, seromucoid, 3 α_1-proteins, 1 of which appears to be the α_{1A}-glycoprotein of Schultze (21), 4 α_2-proteins, including α_2-macroglobulin, α_2-lipoprotein, haptoglobin, and ceruloplasmin, 3 β_1-proteins including β_{1B}- and β_{1C}-globulins, and transferrin, 2 proteins in the β_2-region in the characteristic position of β_{2A}- and β_{2M}-globulins, and gamma globulin were identified. The β_{2M}-arc was quite faint. When macroglobulinemic serum was used as carrier, a strong precipitation line was observed for the β_{2M}-globulin.

[2] The Fe^{59} citrate was supplied by E. R. Squibb and Son, Long Island City, New York.

The rabbit antiserum showed albumin, seromucoid, 2 lines in the α_1-region including α_{1A}-glycoprotein, at least 3 lines in the α_2-region, only 1 of which could be identified (haptoglobin), 4 lines in the β_1-region, 1 of which corresponded to transferrin and another to β_{1C}, leaving 2 unidentified, 2 lines in the β_2-region, 1 corresponding to β_{2A}-globulin, and gamma globulin; a total of 14 lines (Fig. 2).

Both of these antisera developed albumin, α_{1A}-glycoprotein, haptoglobin, transferrin, and gamma globulin lines with monkey serum, and the horse antiserum also showed a clear β_{2M}-line. Several other lines were visible with monkey serum, but could not be clearly distinguished because of antigen excess.

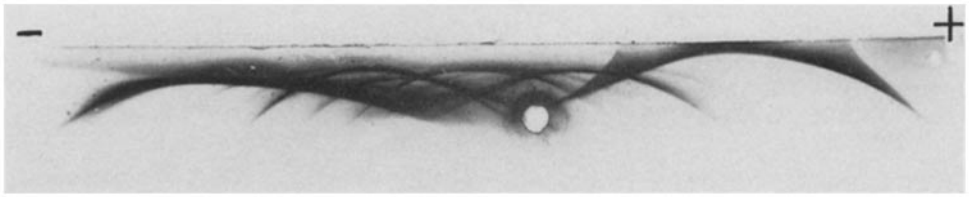

FIG. 2. The immunoelectrophoretic pattern of normal human serum, developed with rabbit anti–whole human serum.

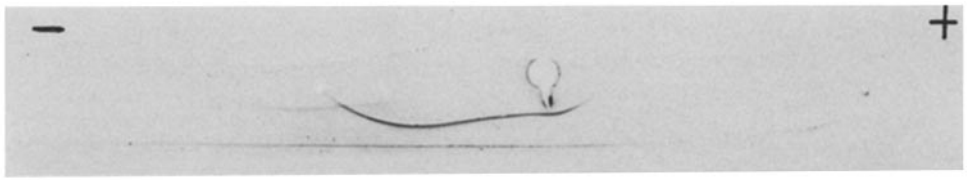

FIG. 3. The immunoelectrophoretic pattern of normal human serum, developed with anti-complement serum.

Rabbit anti-human complement serum gave a line of precipitation corresponding to the β_{1C}-globulin described by Müller-Eberhard (19), with each carrier serum. A strong contaminant line, corresponding to the β_{2M}-globulin was also observed. In addition, there was a weak line in the α_2-region (Fig. 3).

Distribution of Radioactivity in Immunoelectrophoretic Patterns of Various Culture Fluids.—The results with *human tissues* are summarized in Table I. A total of 6 lines, corresponding to gamma globulin, β_{2A}-, β_{2M}-, and β_{1C}-globulins, haptoglobin, and an unidentified α_2-protein, showed radioactivity on analysis of the various culture fluids. Sensitivity for each precipitation arc depended on the relative amounts of both antibody and antigen. For example, the most sensitive combination of antigen and antiserum for detecting labeled gamma globulin proved to be human serum carrier and the rabbit antihuman serum (Fig. 4). With this combination, radioactivity was observed in the gamma globulin line

in analysis of a few tissue culture fluids in which no radioactive gamma globulin was detected with other combinations. Similarly, macroglobulinemic serum carrier with horse antihuman serum as the antiserum was most sensitive in showing radioactivity in the line corresponding to β_{2M}-globulin (Fig. 5). Even with this combination, the labeling of the latter protein was always quite weak. The most sensitive method for the detection of radioactive β_{1C}-globulin proved to be the autoradiography of immunoelectrophoretic patterns of human serum developed with anticomplement serum (Fig. 6). In the culture fluid of one lymph node, which showed stronger radioactivity in the β_{1C}-line than any other culture fluid, the labeling of this protein could be readily detected in the patterns developed with anti-whole human serum.

Labeled β_{2A}- and β_{2M}-globulins were found only in tissue culture fluids which also contained labeled gamma globulin. One fluid contained radioactive β_{2A}-,

TABLE I

Labeling of Serum Proteins by Various Human Tissues In Vitro*

Tissue	Gamma globulin	β_{2A}-globulin	β_{2M}-globulin	β_{1C}-globulin	Haptoglobin	Unidentified α_2-protein
Lymph node	10/13	1/13	6/13	4/13	0/13	2/13
Bone marrow	4/4	4/4	4/4	1/4	4/4	2/4
Ileum	2/2	2/2	1/2	1/2	0/2	0/2
Appendix	1/1	0/1	0/1	0/1	0/1	0/1

* Expressed as the fraction of tissue culture studies showing formation of the specific protein.

but not β_{2M}-globulin; in a few others the reverse was true. Labeled β_{1C}-globulin was found in five culture fluids, one of which showed no other labeled β- or gamma globulins. Table II summarizes the results of analyses of six representative culture fluids, and illustrates the apparent independence of labeling of the various proteins.

Lymph Node.—Of the thirteen culture fluids, three contained radioactive gamma globulin, β_{2M}-globulin, and β_{1C}-globulin; three, labeled gamma globulin and β_{2M}-; one, labeled gamma globulin, β_{2A}-globulin, and β_{2M}-globulin; four, only labeled gamma globulin; and one, labeled β_{1C}-globulin. Two of these culture fluids contained an as yet unidentified labeled α_2-protein. This protein was absent from the immunoelectrophoretic patterns developed with the horse antihuman serum.

Bone Marrow.—Analysis of the four culture fluids showed labeled gamma globulin, β_{2A}-globulin, β_{2M}-globulin, and haptoglobin in each. In addition, one contained labeled β_{1C}-globulin. An unidentified α_2-protein, in the same position

as the one found in the lymph node cultures, was found in two of the culture fluids, including the one containing radioactive β_{1C}-globulin.

TABLE II

Examples of the Patterns of Distribution of Radioactivity in Various Serum Proteins Observed in Individual Culture Fluids of Various Human Tissues

Tissue	Gamma globulin	β_{2A}-globulin	β_{2M}-globulin	β_{1C}-globulin	Haptoglobin	Unidentified α_2 protein
P2 lymph node	+++	—	+	—	—	—
P3 ileum	+++	++	—	—	—	—
P4 lymph node	—	—	—	+	—	+
P13 lymph node	++	—	—	—	—	—
P21 bone marrow	+++	+++	++	++	+++	+
P25 bone marrow	+++	++	++	—	+++	—

Autoradiographic image: the intensity of the autoradiographic image is graded from +++ (very dark) to + (just visible).

TABLE III

Labeling of Serum Proteins by Various Monkey Tissues

Tissue	Albumin	α_{1A}-glycoprotein	Haptoglobin	β_{1C}-globulin	Transferrin	β_{2M}-globulin	Gamma globulin
Spleen	—	—	—	++	—	++	+++
Bone marrow	—	—	++	++	—	++	+++
Lymph node	—	—	—	++	+	++	+++
Lung	—	—	—	+	—	—	+++
Ileum	—	—	—	—	+	++	+++
Liver	++	++	++	—	++	—	+
Colon	—	—	—	—	—	—	+
Thymus	—	—	—	—	—	—	+
Kidney	—	—	—	—	—	—	+
Adrenal	—	—	—	—	—	—	—
Heart	—	—	—	—	—	—	—

Autoradiographic image: the intensity of the autoradiographic image is graded from +++ (very dark) to + (just visible).

Ileum.—Labeled gamma globulin and β_{2A}-globulin were found in both culture fluids. Radioactive β_{2M}- and β_{1C}-globulins were found in one of the two. No other labeled proteins were detected.

Appendix.—The gamma globulin line was weakly labeled in human serum patterns developed with the rabbit antihuman serum. No other labeled lines were found.

The results with *monkey tissues* are summarized in Table III. Three sets of

slides were made with each culture fluid. In one set, isologous monkey serum was used as carrier; in another, normal human serum; and in a third, macroglobulinemic serum. Because some precipitation bands, such as those of albumin and transferrin, were in antigen excess with the antisera used, human serum diluted 1:4 was used as carrier in additional analyses of most of the culture fluids. Human serum carrier was more effective than monkey serum in revealing labeled proteins precipitating in the immunoelectrophoretic patterns. As was the case with the analyses of the human material, macroglobulinemic serum carrier proved best for showing radioactive macroglobulin.

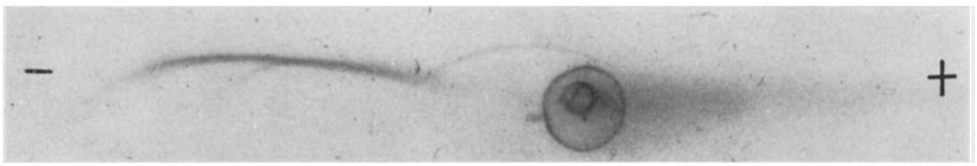

FIG. 4. Photograph of an autoradiograph of the immunoelectrophoretic pattern of bone marrow tissue culture fluid with normal human serum carrier, developed with rabbit antihuman serum. The trailing of radioactive material was seen only with bone marrow cultures.

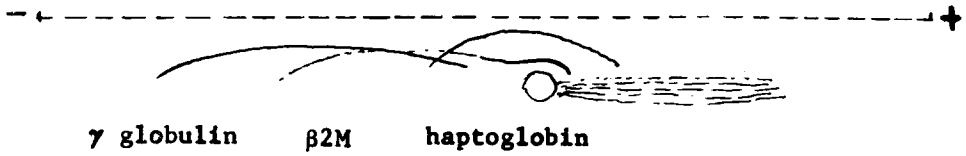

FIG. 5. Tracing prepared from an autoradiograph of the immunoelectrophoretic pattern of bone marrow tissue culture fluid, with macroglobulinemic serum carrier, developed with horse antihuman serum.

A total of 7 lines, representing albumin, α_{1A}-glycoprotein, haptoglobin, transferrin, β_{1C}-globulin, β_{2M}-globulin, and gamma globulin, contained radioactivity upon analysis of the various culture fluids. No significant difference was detected between tissues cultured in the presence of C^{14}-lysine and those cultured in the presence of C^{14}-isoleucine.

Spleen.—The gamma globulin line was the only line labeled when monkey serum was used as carrier. Three lines were labeled in patterns of human serum. In slides developed with the rabbit antihuman serum, the gamma globulin line was strongly labeled. In patterns developed with anticomplement serum, the β_{1C}-line and the line corresponding to the β_{2M}-globulin showed radioactivity. Moreover, the β_{2M}- and gamma globulin lines were labeled when the pattern of macroglobulinemic serum was developed with the horse antihuman serum.

Bone Marrow.—The lines corresponding to gamma globulin and to haptoglobin were the only ones labeled in monkey serum patterns. These lines were also

strongly labeled in patterns made with human serum carrier. As in the spleen, β_{1C}-globulin and β_{2M}-globulin were labeled in the slides using human serum carrier and the anticomplement serum, and the β_{2M}- and gamma globulin lines were labeled in the slides made with marcoglobulinemic serum carrier and the horse antihuman serum.

Liver.—The liver showed much less metabolism than the other tissues. Labeled lines were seen only in patterns developed with antihuman sera. The

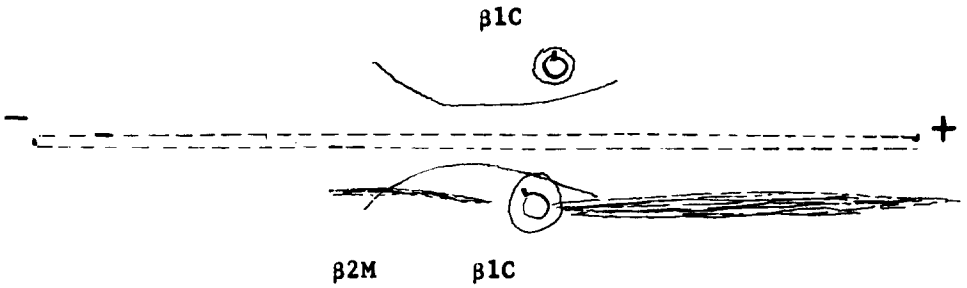

FIG. 6. Tracing prepared from an autoradiograph of the immunoelectrophoretic patterns of normal human serum carrier with lymph node tissue culture fluid (above) and with bone marrow tissue culture fluid (below), developed with an anticomplement serum.

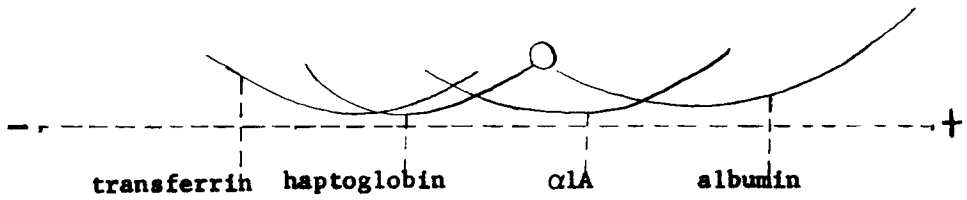

FIG. 7. Tracing prepared from an autoradiograph of the immunoelectrophoretic pattern of monkey liver tissue culture fluid with normal human serum as carrier, developed with rabbit antihuman serum.

slides developed with the horse antihuman serum showed more radioactive lines than those developed with the rabbit antihuman serum, although the more prominent lines showed radioactivity with both antisera. There were four culture fluids: two with C^{14}-isoleucine, and two with C^{14}-lysine. The results with each were slightly different. The only culture fluid of the four showing radioactivity in precipitation lines when monkey serum was used as the carrier antigen was one of the C^{14}-isoleucine cultures. It showed radioactive α_{1A}-glycoprotein, haptoglobin, and transferrin. When human serum was used as the carrier antigen, three of the four culture fluids showed radioactivity in the α_{1A}-glycoprotein, haptoglobin, and transferrin. One of these cultures also showed radioactivity in albumin (Fig. 7). Moreover, weak labeling was found in the gamma

globulin line with two of the four culture fluids (Fig. 8). One of the C^{14}-lysine cultures did not show radioactivity in any of the lines.

Other Organs.—Lung, ileum, lymph node, colon, thymus, and kidney showed labeling of the gamma globulin line with all carrier sera. Of these, only ileum, lymph node, and colon showed incorporation of C^{14}-amino acid into β_2-macroglobulin, detectable with the combination of macroglobulinemic serum and horse antihuman serum. In addition, radioactivity was found in the β_{1C}-line with culture fluids from lung and lymph node, if a combination of normal human serum and anticomplement serum was used to develop the immunoelectrophoretic patterns, and not with other culture fluids. Upon analysis of lymph node and ileum culture fluids, using human serum carrier and rabbit antihuman serum, the transferrin line was found to be weakly labeled. Heart and adrenal

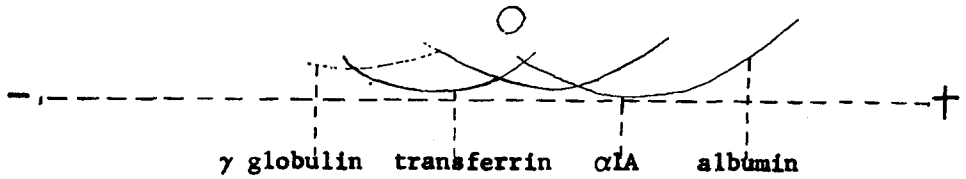

FIG. 8. Tracing prepared from an autoradiograph of the immunoelectrophoretic pattern of monkey liver tissue culture fluid with normal human serum, diluted 1:4, as carrier, developed with horse antihuman serum. Enhanced intensity of the autoradiographic image was obtained by keeping the distance of electrophoretic migration short.

culture fluids did not show radioactivity in any of the immunoelectrophoretic patterns.

DISCUSSION

The results show clearly that gamma globulin, β_2-macroglobulin, and β_{2A}-globulin were all formed in lymph nodes, bone marrow, and in tissue from the intestinal tract in humans, and that gamma globulin and β_2-macroglobulin were formed in these tissues and in spleen of the monkey. Lymphoid tissue seems to be the only tissue common to all of these organs. The serum of the squirrel monkey apparently has no β_{2A}-globulin which cross-reacts sufficiently with human β_{2A}-globulin to be demonstrable with an antihuman serum. Studies with an antiserum prepared against monkey serum should elucidate this.

The method used here to demonstrate the synthesis of these proteins does not readily lend itself to a quantitative estimation of the amounts synthesized. However, for comparable exposure times, the darkness of the autoradiographic image reflects the intensity of radioactivity in the precipitation lines. There was apparently more radioactivity in the β_2-macroglobulin of the bone marrow than of most of the lymph node cultures using this criterion. However, the bone marrow also formed more gamma globulin and β_{2A}-globulin. The weak labeling

of the β_2-macroglobulin in tissue cultures of normal human lymph nodes indicates that the autoradiographic technique used is quite sensitive. Levene *et al.* (14), using isolation of synthesized proteins with a specific antiserum, could demonstrate incorporation of labeled amino acid into β_{2M}-globulin *in vitro* by lymph node tissue only from patients with rheumatoid arthritis.

The finding that gamma globulin, β_2-macroglobulin, and β_{2A}-globulin were formed by lymphoid tissue *in vitro* agrees well with the literature concerning the sites (9, 14) of formation of these proteins in disease states such as rheumatoid arthritis (6, 14), macroglobulinemia (11), and multiple myeloma (10). The only protein about which no studies concerning formation *in vitro* have been reported is the β_{2A}-globulin, but the fact that certain plasmacytomas occur simultaneously with β_{2A}-hyperglobulinemia strongly suggests a similar cellular source for gamma globulin and β_{2A}-globulin (23).

These 3 proteins share antigenic determinants associated with the fragment of gamma globulin carrying the antibody-combining site (24). The fact that each of these proteins contains serum antibodies (25) suggested to some authors (26) the use of the expression "immune globulins" to refer to the 3. The fact that all are formed in similar tissues supports this concept, but it should be kept in mind that the results reported here in no way prove that these "immune globulins" are formed by the same cell or cell type.

The slight labeling of gamma globulin in monkey lung, liver, thymus, and kidney cultures may have been due to the presence of some lymphoid tissue in these organs. It was previously shown (27) that only under conditions where lymphoid tissue with plasma cells are found in the periportal spaces of the rabbit liver, could antibody and gamma globulin formation *in vitro* be demonstrated in that organ, and that gamma globulin formation and lymphoid tissue could regularly be demonstrated in the rabbit lung (28). As histologic examination of the various tissues was not intended in the present study, there is no further evidence for this possibility, as yet. Preliminary studies (29) indicate that cells from the thoracic duct lymph can form all three immune globulins *in vitro*. The capacity for gamma globulin synthesis in such highly vascular organs as kidney and liver might, therefore, reside in the peripheral mononuclear blood elements.

A small percentage (30 per cent) of the human lymph nodes, one of the four bone marrows, and one of the ileums studied, as well as monkey spleen, bone marrow, lymph node, and lung, incorporated labeled amino acid into the β_{1C}-globulin. This protein was shown by Müller-Eberhard *et al.* (19) to represent one of the components of human C'_3. The labeling of the β_{1C}-line was always rather weak. Therefore, its rate of formation *in vitro* appeared to be lower than that of gamma globulin. The labeling of the β_{1C}-protein apparently did not depend on the presence of any labeled other protein in the culture fluids.

The fact that similar tissues seemed to synthesize both the immune globulins

and a component of the complement system may be of interest in the light of recent immunological theories (30). Again, it must be stressed that no evidence has been obtained in the present studies concerning the cell type in lymphoid tissue capable of synthesizing these proteins.

It is known that besides the lymphoid tissue, the liver is the major site of plasma protein formation in the animal. The present studies confirm the fact that the liver is the site of synthesis for albumin (1, 2), α-globulins (1), and transferrin (31). Moreover, the experiments demonstrate the identity of two of the synthesized globulins: haptoglobin and α_{1A}-glycoprotein of Schultze (21).

The results of these experiments agree well with those previously reported for mouse tissues (15). Proteins analogous to the immune globulins and also a protein probably analogous to the β_{1C}-globulin were formed in the mouse spleen. Albumin, a few α-globulins, and transferrin were formed in the mouse liver. Haptoglobin became labeled in cultures of hemopoietic organs, such as mouse spleen, and monkey and human bone marrow. As suggested previously (15), the most likely explanation for the latter finding is that C^{14}-labeled hemoglobin, synthesized by these tissues, combines with haptoglobin in the culture fluids or carrier sera. The results, therefore, do not necessarily reflect haptoglobin formation in these organs. On the contrary, the results obtained with both mouse and monkey liver indicate that this organ is the site of haptoglobin formation.

Besides the liver from both species, lymph node and ileum in the monkey, and spleen in the mouse showed labeled transferrin in the culture fluids. It is possible that reticuloendothelial elements present both in liver and in lymphoid tissue are responsible for its formation. It seems more likely that the labeling of transferrin is due to an as yet unknown carrier function of this protein. Perhaps this phenomenon is related to the known antibacterial properties of transferrin (32).

As was previously observed with mouse tissues, no differences were seen between tissue cultures prepared with C^{14}-isoleucine and with C^{14}-lysine.

Further experiments are planned, in which this approach will be used to study the sites of formation of other serum and plasma proteins, and in which an attempt will be made to elucidate further the cellular source of the immune globulins.

SUMMARY

Human and monkey tissues were cultured in the presence of labeled amino acid. The culture fluids were then examined for labeled serum proteins by means of autoradiography of immunoelectrophoretic patterns prepared from mixtures of the fluids and carrier sera. Gamma globulin, β_{2A}-globulin, β_{2M}-globulin, and β_{1C}-globulin were found to be formed in human lymph node, bone marrow, and ileum. Gamma globulin, β_{2M}-globulin, and β_{1C}-globulin were found

to be formed in monkey spleen, lymph node, and bone marrow. Formation of several other serum proteins was observed in the monkey liver.

BIBLIOGRAPHY

1. Miller, L. L., and Bales, W. F., Synthesis of all plasma proteins except gamma globulin by the liver, *J. Exp. Med.*, 1954, **99,** 125.
2. Peters, T., and Anfinsen, C. B., Production of radioactive serum albumin by liver slices, *J. Biol. Chem.*, 1950, **182,** 171.
3. Askonas, B. A., Humphrey, J. H., and Porter, R. R., On the origin of multiple forms of rabbit gamma globulin, *Biochem. J.*, 1956, **63,** 412.
4. Thorbecke, G. J., Gamma globulin and antibody formation *in vitro*. I. Gamma globulin formation in tissues from young and immature rabbits, *J. Exp. Med.*, 1960, **112,** 279.
5. Miller, G. L., Brown, C. E., Miller, E. E., and Eitelman, E. S., An electrophoretic study on the origin of the abnormal plasma proteins in multiple myeloma, *Cancer Research*, 1952, **12,** 716.
6. Rundles, R. W., Dillon, M. L., Dillon, E. S., and Cooper, G. R., Relation of plasma cell growth to abnormal serum protein components and Bence Jones proteinuria in multiple myeloma, *J. Clin. Inv.*, 1950, **29,** 842.
7. Lane, S. L., Plasmacytoma of the mandible, *Oral Surg. Oral Med. Oral Path.*, 1952, **5,** 434.
8. Ortega, L. G., and Mellors, R. C., Cellular sites of formation of gamma globulin, *J. Exp. Med.*, 1957, **106,** 627.
9. Mellors, R. C., Heimer, R., Corcos, J., and Korngold, L., Cellular origin of rheumatoid factor, *J. Exp. Med.*, 1959, **110,** 875.
10. Vazquez, J. J., Immunocytochemical study of plasma cells in multiple myeloma, *J. Lab. and Clin. Med.*, 1958, **51,** 271.
11. Curtain, C. C., and O'Dea, J. F., Possible sites of macroglobulin synthesis: a study made with fluorescent antibody, *Australasian Ann. Med.*, 1959, **8,** 143.
12. Martin, C. M., Waite, J. B., and McCoullough, N. B., Antibody protein synthesis by lymph nodes homotransplanted to a hypogammaglobulinemic adult, *J. Clin. Inv.*, 1957, **36,** 405.
13. Meyer, F., C^{14}-lysine incorporation into Bence Jones protein by cultures of myeloma bone marrow, *Fed. Proc.* 1957, **16,** 222.
14. Levene, H., Franklin, E. C., and Thorbecke, G. J., *In vitro* formation of 7 S and 19 S gamma globulin by tissues from patients with rheumatoid arthritis, *J. Immunol.*, 1961, **86,** 440.
15. Hochwald, G. M., Thorbecke, G. J., and Asofsky, R., Sites of formation of immune globulins and a component of C'_3 I. A new technique for the demonstration of the synthesis of individual serum proteins by tissues *in vitro*., *J. Exp. Med.*, 114, 459.
16. Williams, C. A., and Wemyss, C. T., Experimental and evolutionary significance of similarities among serum protein antigens of man and the lower primates, *Ann. New York Acad. Sc.*, in press.
17. Eagle, H., Oyama, V. I., Levy, M., Horton, C. L., and Fleischman, R., The

growth response of mammalian cells in tissue culture to L-glutamine and L-glutamic acid, *J. Biol. Chem.*, 1956, **218**, 607.

18. Neuman, R. E., and McCoy, T. A., Growth-promoting properties of pyruvate oxalate and α-ketoglutarate for isolated Walker carcinoma 256 cells, *Proc. Soc. Exp. Biol. and Med.*, 1956, **98**, 303.
19. Müller-Eberhard, H. J., Nillson, U., and Aronsson, T., Isolation and characterization of two β-glycoproteins of human serum, *J. Exp. Med.*, 1960, **111**, 201.
20. Burtin, P., Les protéines du plasma humain normal, *in* Analyse Immunoélectrophorétique, (P. Grabar and P. Burtin, editors), Paris, Masson et Cie, 1960, 33.
21. Schultze, H. E., Gollner, I., Heide, K., Schönenberger, M., Schwick, G., Zur Kenntnis der α-globuline des menschlichen Normalserums, *Z. f. Naturw.*, 1955, **10b**, 463.
22. Uriel, J., Les réactions de caractérisation des constituants protéiques après électrophorèse ou immunoélectrophorèse en gélose, *in* Analyse Immunoélectrophorétique, (P. Grabar and P. Burtin, editors), Paris, Masson et Cie, 1960, 33.
23. Heremans, J. F., Heremans, M. T., and Schultze, M. E., Isolation and description of a few properties of the β_{2A}-globulin of human serum, *Clin. Chem. Acta.*, 1959, **4**, 96.
24. Franklin, E. C., and Stanworth, D. R., Studies on the antigenic specificity of human 7 S gamma globulin, *Fed. Proc.*, 1961, **20**, 31.
25. Schultze, H. E., The synthesis of antibodies and proteins. 7th Colloquium on Protides of the Biological Fluids, Brussels, 1959, *Clin. Chem. Acta.*, 1959, **4**, 610.
26. Heremans, J. F., Immunochemical studies on protein pathology: the immunoglobulin concept, *Clin. Chem. Acta.*, 1959, **4**, 639.
27. Thorbecke, G. J., and Keuning, F. J., Antibody and gamma globulin formation *in vitro* in hemopoietic organs, *J. Infect. Dis.*, 1956, **98**, 157.
28. Thorbecke, G. J., Formation of gamma globulin *in vitro* by tissues taken from newborn, immature, and adult rabbits, *in* Mechanisms of Antibody Formation, Prague, Czechoslovak Acad. Sc., 1960, 297.
29. Dumont, A., Asofsky, R., and Thorbecke, G. J., data to be published.
30. Szilard, L., The molecular basis of antibody formation, *Proc. Nat. Acad. Sc.*, 1960, **46**, 293.
31. Clausen, J., Rask-Nielsen, R., Christensen, H. E., and Munkner, T., Two transplantable mouse hepatomas associated with an increase of metal combining β-globulin (transferrin) in serum, *Cancer Research*, 1960, **20**, 178.
32. Osserman, E. F., and Kohler, P. F., Demonstration of an antibacterial serum protein constituent of alpha 2: beta mobility by a new technique of protein electrophoresis in agar gell impregnated with bacteria, *Fed. Proc.*, 1961, **20**, 74.