increase rapidly after 36 hr. until growth is completed in 5–6 days (Goodwin & Osman, 1953). In 24–36 hr. cultures, spirilloxanthin represents only about 20–30 % of the total pigments present. Its increased rate of synthesis in older cultures, coupled with the gradual disappearance of the other pigments, results in it representing 92 % of the total pigments, in 5- to 6-day cultures. This agrees with previous observations (Polgár *et al.* 1944; Goodwin & Osman, 1953). The remaining 8 % is made up of P 481 and hydroxy-P 481, lycopene and lycoxanthin having disappeared completely after 72–96 hr. This differential rate of synthesis among the carotenoids parallels the situation in cultures of *Phycomyces blakesleeanus* inhibited by diphenylamine (Goodwin, Jamikorn & Willmer, 1953); in this fungus, the rate of synthesis of the phytofluene series is high in young cultures but falls off rapidly with age, whereas that of β -carotene reaches its maximum only in older cultures.

SUMMARY

1. The problem of carotenoids and phototaxis in *Rhodospirillum rubrum* is discussed.

2. Carotenoids present in young *Rsp. rubrum* cultures are lycopene (rhodopurpurene), spirilloxanthin, lycoxanthin (rhodopin), demethylated spirilloxanthin, pigments P 481 and hydroxy-P 481 (? rhodovibrin).

3. The relative amounts of the constituent pigments present in cultures of various ages are recorded. Spirilloxanthin gradually increases throughout the growing period. All the other components reach a maximum at about 24 hr. and thereafter decline, some being completely absent from 72–96 hr. cultures.

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On the Origin of the Multiple Forms of Rabbit γ -Globulin

By B. A. ASKONAS, J. H. HUMPHREY AND R. R. PORTER
National Institute for Medical Research, The Ridgeway, London, N.W. 7

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γ -Globulin is complex biologically in that it may contain many different antibodies; it is complex physically, as is shown, for example, during electrophoresis, and in certain species chemical complexity is apparent from the varying terminal amino acids. Most protein preparations show some degree of heterogeneity when examined with sufficient care (Colvin, Smith & Cook, 1954), but the complexity of

γ -globulin appears to be altogether greater than that found in well-characterized proteins.

It has been found that resolution of rabbit γ -globulin can be effected by partition chromatography and that the distribution of antibodies, among the fractions obtained, depends on the route and duration of the antigenic stimuli (Porter, 1955; Humphrey & Porter, 1956). It became apparent

that an understanding of antibody formation and the relation of antibodies to inert γ -globulin would be facilitated if the reasons for the diversity of γ -globulin could be found. Three possible explanations have been suggested (Porter, 1955): (1) Different cells in the same or different tissues produce slightly varying types of globulin. (2) The system responsible for γ -globulin synthesis is sensitive to changes in environmental conditions so that small differences in end product would result in response to such changes. (3) γ -Globulin has a labile structure such as that proposed by Pauling (1940) and would be expected to show heterogeneous physical properties with the majority of the molecules assuming the most stable configurations.'

These possibilities were investigated by studying the rate of incorporation of radioactive amino acids into the different chromatographic fractions of γ -globulin.

If Pauling's suggestion that γ -globulin is produced with a labile configuration and then takes up a variety of stable configurations is correct, it might be expected that on injection of labelled amino acids into a rabbit one fraction would become radioactive rapidly, and the labelling would then spread into other fractions as conversion occurred. Such a conversion might be missed if it occurred very rapidly, and would be undetected if it went on during bleeding and subsequent isolation of the γ -globulin, or if it took place in the cell before release of the γ -globulin into the vascular system.

In a similar experiment, if the second suggestion, that cells making γ -globulin are sensitive to environmental changes and this results in a slightly different end product at different times, is correct, it would be expected that after injection of labelled amino acids certain fractions would become labelled first and others later in an uneven manner until all had the same specific activity. The order in which the different fractions become labelled might vary from experiment to experiment.

It was found that with the exception of the fast-running fraction the specific activity of the fractions was the same, and increased from nil at 30 min. after injection to a maximum at about 6 hr., from which it slowly fell off. This makes it improbable that the different end products could be produced at different times, but possible that the fast-running fraction which became radioactive much more rapidly than the rest might be a precursor. Direct test failed to support this, however, and further work showed that the more rapid incorporation into the fast fraction was due to contamination of this fraction with β -globulin.

The possibility that different cells might be producing their characteristic type of γ -globulin was investigated by following the rate of incorporation of radioactive amino acids into the fractions

when slices of different tissues were incubated *in vitro* in their presence (Askonas & Humphrey, 1955; Askonas & White, 1956). It was found that although some variation from animal to animal occurred the spleen incorporated relatively more radioactivity into the middle fractions, and the lymph nodes and bone marrow more into the slower-moving fractions. Hence the first explanation offered for heterogeneity of γ -globulin seemed to be the most probable.

MATERIALS AND METHODS

Administration of radioactive amino acids. All radioactive amino acids were purchased from the Radiochemical Laboratory, Amersham. Those used were [^{14}C]glycine, [^{14}C]-L-valine, [^{35}S]-DL-methionine and acid-hydrolysed algal protein prepared from algae grown in an atmosphere of $^{14}\text{CO}_2$ (Catch, 1954). For intravenous injection the isotopic amino acids were dissolved in 1 ml. of 0.9% NaCl and neutralized if necessary.

Immunization of animals. Rabbits were immunized intravenously with alum-precipitated ovalbumin, as has been described (Porter, 1955).

Preparation and chromatography of γ -globulin. In all experiments 10 mg. of the appropriate inactive amino acids was added per ml. of serum or solution, before isolation of the γ -globulin. All buffers used in the salting-out procedure and in dialysis contained 1 mg. of inactive amino acid per ml. This effectively diluted out any radioactive amino acids remaining adsorbed to the protein, control experiments showing that there was a negligible contamination under these conditions.

Rabbit γ -globulin was prepared by the Na_2SO_4 precipitation method (Porter, 1950) described for human γ -globulin by Kekwick (1940).

Zone electrophoresis was carried out in a column (40 cm. $\times$ 2.6 cm.) with treated cotton wool as supporting medium according to published methods (Flodin & Porath, 1954; Porath, 1954; Campbell & Stone, 1956). The borate-phosphate buffer (pH 8.6) described by these authors was used, with a voltage of 350 v and a current of 30 ma.

Partition chromatography of rabbit γ -globulin was carried out as previously described (Porter, 1955; Humphrey & Porter, 1956) with 40 g. of washed and graded Celite 545 (Johns-Manville and Co. Ltd., London, S.W. 1) in a jacketed column having an internal diameter of 2.2 cm.

Elution of protein from the column was followed by measurement of the absorption at 280 $\mu\mu$. in a 1 cm. cell in a spectrophotometer. The eluates obtained after chromatography of about 60 mg. of freeze-dried γ -globulin were combined to yield 8-9 fractions; these were concentrated by dialysis under 1 atm. at 2°.

The protein fractions were prepared for counting by precipitation of dialysed solutions with 2 vol. of ethanol at room temperature. After 1 hr. the precipitate was centrifuged down and washed once with a mixture of ethanol and ether (1:1, v/v) and then twice with ether. The ether remaining in the precipitate was removed *in vacuo*. When [^{35}S]methionine was being used protein samples were also treated with performic acid to remove thiol-bound impurities before counting (Peterson & Greenberg, 1952).

Incorporation of radioactive amino acids into γ -globulin in vitro. The rabbits were anaesthetized by injecting 2 ml. of a 0.6% solution of Nembutal. Slices of various organs were incubated in an isotonic salt medium in the presence of ^{14}C -labelled amino acids for 2.5 hr. according to the method described by Askonas & Humphrey (1955) and Askonas & White (1956). The labelled γ -globulin was then extracted by homogenizing the tissue slices in the presence of inactive amino acid and 2 ml. of the rabbit's own serum. A portion (10 ml.) of the rabbit's own serum was then added, and the γ -globulin was prepared by Na_2SO_4 precipitation, dialysed and freeze-dried before chromatography.

Determination of radioactivity. For measuring the radioactivity of the labelled protein fractions two methods were used. In some experiments with ^{14}C -labelled amino acids a proportional counter was used after conversion of all carbon into CO_2 (Bradley, Holloway & McFarlane, 1954). In other experiments with ^{14}C -labelled amino acids and in all experiments with ^{35}S -labelled amino acids the fractions were counted on 0.3 or 1 sq. cm. disks at infinite thickness. An automatic-recording Geiger counter with a thin end-window was used.

Immunological examination. Immunological examination of the chromatographic fractions, for antigenic heterogeneity, was carried out by Oakley's modification (Oakley & Fulthorpe, 1953) of Oudin's (1946) method of diffusion in agar gel.

RESULTS

Rate of synthesis of γ -globulin fractions in vivo

A rabbit was injected intravenously with 100 μC (1.5 mg.) of an acid hydrolysate of ^{14}C -labelled algal protein. After 1, 4 and 24 hr. about 40 ml. of blood was taken, the serum collected and γ -globulin prepared from it. About 60 mg. of the freeze-dried preparations was chromatographed and the eluted protein divided into eight or nine fractions. The radioactivity of the fractions was measured by gas counting. Figs. 1-3 show the results obtained at 1, 4 and 24 hr. respectively. At 1 hr. there is a sharp peak of radioactivity running right at the front, but the greater part of the γ -globulin has a low uniform radioactivity. At 4 hr. a similar result was obtained, though the differences between the specific activities of the first and other fractions are reduced, and at 24 hr. the activity of the fast fraction has fallen, while that of the main part is higher and differences are now slight.

As we were injecting ^{14}C -labelled amino acids it seemed possible, however, that our results might be due to preferential labelling of fat or carbohydrate that was perhaps concentrated in the fast-running fraction. The experiment was therefore repeated with [^{35}S]-DL-methionine in place of ^{14}C -labelled amino acids. Figs. 4-6 show that similar results were obtained when the rabbit was bled after 30 min., 1 and 4 hr. After 30 min. the specific activity of most of the γ -globulin was nil, but that of the fast fraction was relatively high.

Fig. 1. Specific activity of γ -globulin fractions 1 hr. after injection of 100 μC of hydrolysed ^{14}C -labelled algal protein into a rabbit. About 60 mg. of γ -globulin chromatographed on a column of 40 g. of Celite 545. Internal diameter 2.2 cm. $\times$ — $\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at 280 $\text{m}\mu$; $\bullet$ — $\bullet$, specific activity.

Fig. 2. Specific activity of γ -globulin fractions 4 hr. after injection of 100 μC of hydrolysed ^{14}C -labelled algal protein. Conditions as in Fig. 1. $\times$ — $\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at 280 $\text{m}\mu$; $\bullet$ — $\bullet$, specific activity.

Fig. 3. Specific activity of γ -globulin fractions 24 hr. after injection of 100 μC of hydrolysed ^{14}C -labelled algal protein. Conditions as in Fig. 1. $\times$ — $\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at 280 $\text{m}\mu$; $\bullet$ — $\bullet$, specific activity.

Heterogeneity of fast-running fraction

It seemed possible that the fast fraction might be a precursor of the other γ -globulin fractions. However, it was also possible that this fraction contained proteins other than γ -globulin which, though synthesized more rapidly, were unrelated to it. Earlier, examination of the rabbit γ -globulin, prepared by Na_2SO_4 precipitation, by either free electrophoresis or zone electrophoresis on paper, had failed to show any significant impurity. Now 200 mg. of γ -globulin

was fractionated by zone electrophoresis on a treated cotton-wool column as described above, and under these conditions it was apparent that the preparation contained small amounts (less than 5%) of β -globulin. The rate of uptake of amino acids into the different serum globulins was therefore studied. A rabbit was injected with radioactive amino acids and bled 4 hr. later. A portion (3 ml.) of serum was subjected to electrophoresis for 40 hr. at about 7 v/cm. with a current of 30 mA. Under these conditions the albumin moves off the column and the maximum separation of the globulin is obtained. After elution the radioactivity of samples from different globulin fractions was estimated. The results are shown in Fig. 7, and it can be seen that

Fig. 5. Specific activity of γ -globulin fractions 1 hr. after injection of 350 μC of [^{35}S]-DL-methionine as in Fig. 4. $\times-\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at 280 m μ ; $\bullet-\bullet$, specific activity.

Fig. 4. Specific activity of γ -globulin fractions 30 min. after injection of 350 μC of [^{35}S]-DL-methionine into a rabbit. Chromatography as in Fig. 1. $\times-\times$, Protein concentration expressed as $E_{1\text{ cm.}}$ at 280 m μ ; $\bullet-\bullet$, specific activity.

Fig. 6. Specific activity of γ -globulin fractions 4 hr. after injection of 350 μC of [^{35}S]-DL-methionine as in Fig. 4. $\times-\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at 280 m μ ; $\bullet-\bullet$, specific activity.

Fig. 7. Specific activity of serum-globulin fractions after separation by zone electrophoresis on treated cotton-wool column (40 cm. $\times$ 2.6 cm.). Serum taken 4 hr. after injection of hydrolysed ^{14}C -labelled algal protein into a rabbit. $\times-\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at 280 m μ ; hatched bar , specific activity.

the specific activities of the α - and β -globulins are considerably higher than those of the γ -fractions.

β -Globulin, isolated by zone electrophoresis, was chromatographed under the same conditions as those used for γ -globulin; it was found to move fast, with only a slight trail, showing that any β -globulin impurities would be in the fast fraction. Hence it was concluded that this rapidly synthesized fraction in our γ -globulin preparations probably arose from β -globulin impurities. Further evidence of the heterogeneity of this fast fraction was obtained from use of Oudin's technique, where antigen and antiserum are allowed to diffuse towards each other in an agar gel. The slower fractions showed evidence of only one antigenic component, and the fastest contained two additional components.

Interconversion of γ -globulin fractions

Although it seemed most probable that the rapidly synthesized fraction arose from β -globulin, it could not be completely excluded that this fraction might be a precursor of the others. Hence a direct test of extracellular conversion was made. An acid hydrolysate (1 mc, 16 mg.) of algal protein was injected into a rabbit, and 4 hr. later the animal was anaesthetized with Nembutal and bled out as completely as possible. A suitable volume (170 ml.) of blood was obtained, and the γ -globulin prepared and fractionated in a series of chromatograms. The fast fractions from each were combined, concentrated and dialysed to give 10 mg. of protein with a specific activity of about 12 000 counts/min./mg. of C in a proportional counter. This material was dissolved in 1 ml. of 0.9% NaCl and injected into a second rabbit; 30–40 ml. of blood was removed after 5 min., 4, 24 and 48 hr. The γ -globulin was prepared and chromatographed, and the radioactivity of the fractions estimated.

The results at the four times were the same. The activity remained confined almost entirely to the fast-running fractions; only a slight spread into the next fraction occurred, and this may be attributed to the chromatographic impurity of the material injected. Unless alteration of the fast fraction has occurred during the preparation, it is clear that it cannot be an extracellular precursor of the other fractions.

A further test was made to determine whether inert γ -globulin from one rabbit could be converted into antibody in a second rabbit undergoing active immunization. A portion (20 mg.) of a slower inert fraction with an activity of 6000 counts/min./mg. of C was injected into a rabbit which was receiving injections of alum-precipitated ovalbumin. At different time intervals up to 72 hr. after injection, blood samples (5 ml.) were taken, the antibody was precipitated with the antigen and the radioactivity of the specific precipitate estimated. Although

rapid synthesis of antibody was occurring (as shown by the maintenance of a steady high level of antibody in the plasma), at no time was any significant activity found in the antigen-antibody precipitate, showing that no conversion of globulin into antibody could have occurred.

Incorporation of radioactive amino acids into γ -globulin fractions by tissues in vitro

As γ -globulin is secreted into the vascular system it is difficult to assess the contribution of individual organs by experiments carried out in the living animal. Advantage was therefore taken of a recently developed method (Askonas & Humphrey, 1955; Askonas & White, 1956) of studying the rate of uptake of amino acids into γ -globulin when tissue slices are incubated *in vitro*. The slices of tissues from a stock rabbit were incubated in a medium containing 7.5 μ C of [14 C]-L-valine at 37°. After 2.5 hr. the tissue and incubation medium were homogenized and centrifuged. In order to get sufficient material for fractionation the rabbit's own serum was added and the γ -globulin prepared from this solution. It was chromatographed as described above and the results are shown in Figs. 8–11. As the radioactive γ -globulin formed was diluted similarly for each tissue, any differences in the labelling of the fractions will indicate that the tissues used have different synthetic abilities.

The finding that the kidney (Fig. 8) has not incorporated any significant radioactivity into the γ -globulin is in agreement with Askonas & White (1956), who showed that kidney took no part in the synthesis of antibody and γ -globulin in the subcutaneously immunized guinea pig. In the spleen (Fig. 9) there are obvious differences in the specific activity of the different fractions, the central fractions being more active than the slower fractions. The bone marrow and lymph nodes show the reverse effect, suggesting that the different tissues are synthesizing different fractions at different rates. In all cases there is high activity in the first fractions, but for reasons discussed above it is considered that this high radioactivity arises from impurities and not from γ -globulin. The satisfactory blank given by kidney suggests that any cellular proteins in the homogenate have been eliminated in the fractionation procedures.

This experiment was repeated with organs from a rabbit which had been immunized with alum-precipitated ovalbumin. The animal had received nine intravenous injections in 3 weeks, and 3 months later a further six injections were given, the last being administered 2 days before the experiment.

The conditions used were identical with those above except that 6 μ C of [14 C]glycine was added to each incubation flask instead of 7.5 μ C of [14 C]-L-

Fig. 8. Specific activity of γ -globulin fractions after incubation *in vitro* of kidney slices (1 g.) in the presence of $7.5 \mu\text{c}$ of $[^{14}\text{C}]\text{-L-valine}$. Chromatography as in Fig. 1. $\times\text{---}\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at $280 \text{ m}\mu$; $\bullet\text{---}\bullet$, specific activity.

Fig. 9. Specific activity of γ -globulin fractions after incubation *in vitro* of spleen slices (1 g.) in the presence of $7.5 \mu\text{c}$ of $[^{14}\text{C}]\text{-L-valine}$. Chromatography as in Fig. 1. $\times\text{---}\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at $280 \text{ m}\mu$; $\bullet\text{---}\bullet$, specific activity.

Fig. 10. Specific activity of γ -globulin fractions after incubation *in vitro* of slices of cervical lymph nodes (1 g.) in the presence of $7.5 \mu\text{c}$ of $[^{14}\text{C}]\text{-L-valine}$. Chromatography as in Fig. 1. $\times\text{---}\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at $280 \text{ m}\mu$; $\bullet\text{---}\bullet$, specific activity.

Fig. 11. Specific activity of γ -globulin fractions after incubation *in vitro* of bone marrow (1 g.) in presence of $7.5 \mu\text{c}$ of $[^{14}\text{C}]\text{-L-valine}$. Chromatography as in Fig. 1. $\times\text{---}\times$, Protein concentration, expressed as $E_{1\text{ cm.}}$ at $280 \text{ m}\mu$; $\bullet\text{---}\bullet$, specific activity.

valine. Also two incubation mixtures with slices of spleen tissue were set up in order to compare the behaviour of γ -globulin before and after removal of antiovalbumin by precipitation with ovalbumin. Figs. 12–15 show that the results were very similar to those obtained with tissues from a non-immunized rabbit. With lymph nodes and bone marrow the slow fractions become labelled more rapidly, but the spleen incorporates radioactivity considerably faster into the middle fractions than into the slower ones. Fig. 15 shows the result with spleen slices when γ -globulin, from which antibody had been removed, was fractionated. By comparison with Fig. 14, where the γ -globulin was run without prior precipitation of the antibody, it is apparent that the antiovalbumin being synthesized by the spleen is largely confined to the middle fractions.

DISCUSSION

If the protein-elution curves in the different chromatograms illustrated in this paper are compared, it will be observed that the R value of the fraction with the highest protein concentration varies somewhat. There are also differences in the shapes of the curves. These chromatograms have been obtained from experiments extending over 1 year, and it is considered that the differences do not reflect variation in the composition of the γ -globulin. They arise almost certainly from the sensitivity of the phase system to very slight changes of composition and of temperature. Such chromatograms are exactly reproducible while the same column is being used or when the greatest care is taken to reproduce the conditions exactly, but in routine use some variation seems inevitable. It should have little influence on the effectiveness of the fractionation.

It will also be seen that the fast-running peak is greater in the chromatograms of γ -globulin from the experiments *in vitro*. The values noted for the protein concentration in these fractions are not accurate, as the samples frequently contain pigment. Any pigment persisting in these preparations from homogenized tissue is confined to this fast-running peak; all the remaining fractions give a satisfactory ratio for the absorption at 260 and 280 $m\mu$. For reasons discussed above it is considered that this fraction contains impurities not related to γ -globulin, and it has been disregarded in the present discussion.

Fig. 12. Specific activity of γ -globulin fractions after incubation *in vitro* of cervical lymph nodes (1 g.) from an immune rabbit in the presence of $6\mu\text{C}$ of $[^{14}\text{C}]$ -glycine. Chromatography as in Fig. 1. $\times$ — $\times$, Protein concentration, expressed as $E_{1\text{ cm. at } 280\text{ m}\mu}$; $\bullet$ — $\bullet$, specific activity.

Fig. 13. Specific activity of γ -globulin fractions after incubation *in vitro* of bone marrow (1 g.) from an immune rabbit in the presence of $6\mu\text{C}$ of $[^{14}\text{C}]$ -glycine. Chromatography as in Fig. 1. $\times$ — $\times$, Protein concentration, expressed as $E_{1\text{ cm. at } 280\text{ m}\mu}$; $\bullet$ — $\bullet$, specific activity.

The results of the experiments carried out *in vivo* show that from 30 min., when labelled γ -globulin first appears, to 24 hr., when the activity is falling, the amino acids have been incorporated into all fractions at the same rate. Therefore there can be no simple conversion in the blood of a precursor into other forms. The possibility that material in the fast-moving fraction could be a precursor of the other fractions was excluded by direct test. It was also shown that γ -globulin from appropriate fractions was not converted into antibody when injected into a second rabbit which was synthesizing antibody. It is possible that such a conversion could occur in the cells making γ -globulin before its release into the blood, or that the conversion is so rapid after release that it was undetected by the methods used or, again, that the conversion took place outside the body after bleeding.

Fig. 14. Specific activity of γ -globulin fractions after incubation *in vitro* of spleen slices (1 g.) from an immune rabbit in the presence of $6\mu\text{C}$ of $[^{14}\text{C}]$ -glycine. Chromatography as in Fig. 1. $\times$ — $\times$, Protein concentration, expressed as $E_{1\text{ cm. at } 280\text{ m}\mu}$; $\bullet$ — $\bullet$, specific activity.

Fig. 15. Specific activity of γ -globulin fractions after incubation *in vitro* of spleen slices (1 g.) from an immune rabbit in the presence of $6\mu\text{C}$ of $[^{14}\text{C}]$ -glycine. Antibody was precipitated from the γ -globulin, before chromatography. $\times$ — $\times$, Protein concentration, expressed as $E_{1\text{ cm. at } 280\text{ m}\mu}$; $\bullet$ — $\bullet$, specific activity.

The uniform labelling of all fractions in several experiments makes it unlikely that cells produce different types of γ -globulin at different times.

In the experiments carried out *in vitro* slices of different organs incorporated radioactive amino acids into the different fractions of γ -globulin at varying rates. Askonas & White (1956) have given reasons for believing that the rate of incorporation of activity is a measure of the synthetic ability of the tissues, and this is supported by other experiments (Humphrey & Sulitzean, unpublished work) in which it was shown that a measure of the rate of antibody synthesis *in vivo* by different organs of an actively immunized rabbit gave results similar to those obtained *in vitro*.

It therefore seems justified to conclude that different tissues make the various γ -globulin fractions at different rates, the spleen forming the middle fractions relatively faster, compared with the lymph nodes and bone marrow, which are making the slower fractions more rapidly. From the results with an actively immunized rabbit it appears that the pattern of synthesis by different tissues is the same as in the normal animal. Again, the spleen was shown to synthesize the middle fractions relatively more rapidly (particularly the antibody) and the lymph nodes and bone marrow the slower fractions more quickly. Some variation in detail occurs, but in other similar experiments the same contrast between spleen on one hand and lymph nodes and bone marrow on the other was found. As all the tissues examined synthesize all the fractions, though at different rates, the simplest explanation appears to be that individual cells are synthesizing characteristic types of γ -globulin, and that the distribution of these cells is different in the various organs. Certain types predominate in the spleen and others predominate in the lymph nodes and bone marrow.

These results suggest an explanation of the earlier observation (Porter, 1955; Humphrey & Porter, 1956) that when a rabbit is immunized by intravenous injections the antibodies are at first confined to the slower-running fractions, and then after prolonged injections they appear more and more in the middle fractions. Such a result would be explicable if the lymph nodes or bone marrow, or both, were very active in antibody production in the earliest stages of immunization, and if the spleen became a relatively more important site of antibody production as immunization continued. A preliminary experiment was carried out in which the relative rates of antibody synthesis in slices of the different organs from rabbits in the early and late stages of immunization were compared (Askonas & Humphrey, unpublished work). It showed that such a shift in relative rates of antibody formation in lymph nodes, bone marrow and spleen did in fact occur.

SUMMARY

1. The rate of incorporation of radioactive amino acids into the different chromatographic fractions of rabbit γ -globulin has been investigated.

2. It has been shown by experiments *in vivo* that there is no evidence for conversion of one form of γ -globulin into another, or of γ -globulin into antibody, in the vascular system.

3. Experiments *in vitro* showed that amino acids are incorporated into different fractions of γ -globulin at different rates, there being an obvious difference between the spleen and the various lymph nodes and bone marrow which were tested.

4. It is suggested that different types of γ -globulin are synthesized by different cells which are unevenly distributed in the tissues making γ -globulin.

5. The observation that different tissues synthesize different fractions of γ -globulin at different rates may in turn explain the earlier observation that chromatographically different antibodies appear at successive stages in the immunization of a rabbit.

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