In contrast, the exponential decay of an enzyme protein *in vivo* is a process resulting from the dynamic synthesis and degradation of proteins and presumably independent of the stability of its RNA template. For example, the half life of the enzyme serine dehydrase is about 3 hr, whereas the lifetime of the template RNA for serine dehydrase appears to be at least 6–8 hr.

[18] Penman, S., K. Scherrer, Y. Becker, and J. E. Darnell, these PROCEEDINGS, **49**, 654 (1963).
[19] Noll, H., T. Staehelin, and F. O. Wettstein, *Nature*, **198**, 632 (1963).
[20] Pitot, H. C., *Perspectives Biol. Med.*, **8**, 50 (1964).
[21] Pitot, H. C., and C. Peraino, *Biochem. Clinics*, **3**, 139 (1964).

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*THE EFFECT OF PROGRESSIVE IMMUNIZATION ON POLYRIBOSOMAL SIZE IN LYMPHOID CELLS**

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Although the existence of a variety of immunoglobulins is well recognized, the factors which determine their production have not been completely clarified. Each of the immunoglobulins contains immunologically similar subunits, referred to as light or L chains, and immunologically distinct subunits, referred to as heavy or H chains.[1] Of particular interest is the relationship between IgM (19*S*) and IgG (7*S*) antibodies, since the usual response to most antigens is characterized by early 19*S* antibody production which subsequently gives way to predominantly 7*S* antibody production.[2] Moreover, the presence of 7*S* antibody may inhibit the induction of 19*S* antibody.[3] In attempting to gain some understanding of these phenomena, it seemed worth while to investigate certain parameters of normal protein formation which pertain to the synthesis of the different immunoglobulins. Since polyribosomes are the site of protein synthesis, their characteristics in cells producing specific 19*S* and 7*S* antibodies were examined.

The characterization of polyribosomal aggregates with respect to size and amino acid incorporation has been achieved in a number of biological systems.[4–7] Since the maximum size of the polyribosome is thought to be determined by the length of its messenger RNA, determination of the degree of ribosomal aggregation may provide indirect evidence regarding messenger length and hence the size of synthetic subunits of a particular protein.[4]

Polyribosomal aggregates from lymphoid tissue with sedimentation coefficients of 110*S* and 130*S* have been described.[8, 9] More recently, 200*S* polyribosomes from rabbit lymph nodes have been detected.[10] In these published reports, immunization programs were employed which would be expected to elicit the predominant production of 7*S* antibody. It is the purpose of this communication to describe the polyribosomal population of rabbit lymph node cells producing specific 19*S* antibody, and others producing specific 7*S* antibody.

Since a variety of proteins may be synthesized by lymphoid tissue, it is desirable to demonstrate that the polyribosomes studied are, in fact, associated with immunoglobulin synthesis. Therefore, preliminary experiments on the immuno-

logic identification of nascent gamma globulin on polyribosomes will also be reported.

Materials and Methods.—Young adult, giant albino rabbits were immunized with alum-precipitated hen egg albumin (EA), alum-precipitated sulfanilyl bovine serum albumin (sulfanilyl BSA), and typhoid 0 antigen. Immunization was carried out by injection into the footpads at 2–3-day intervals for a total of 1–6 injections. Animals receiving EA had received primary immunization in Freund's adjuvant 1–2 months prior to injection of the alum precipitate.

Cell suspensions prepared from regional lymph nodes, and containing approximately 300 × 10^6 cells, were incubated at 37°C in 2 ml of modified Eagle's medium containing 10% of the usual amino acid content. After 15 min, 20 μc/ml of ULC[14] algal hydrolysate was then added to each sample. After 2–5 min, the cells were washed with cold buffer A of Warner and co-workers[4] and homogenized in 2 ml of buffer B of the latter workers. These and subsequent procedures were carried out at 4°C. Buffers contained bentonite throughout. After centrifugation at 1,000 *g* for 20 min, 0.1 vol of 5% sodium deoxycholate solution was added to the supernatant, which was then chromatographed on Sephadex G-25 or G-100. Two milliliters of the first optical density peak measured at 260 mμ was layered on a 30-ml, 5–30% (w/v) sucrose density gradient and centrifuged in a Spinco SW 25.1 head at 25,000 rpm for 2 hr. Equal fractions were collected, and each fraction was counted directly or precipitated by adding 4 vol of cellosolve after the addition of 4 mg of cold carrier ribonucleic acid. The precipitates were dissolved in 1 ml of buffer B, and counted in Bray's phosphor with thixotropic gel[11] in a Packard Tricarb scintillation counter.

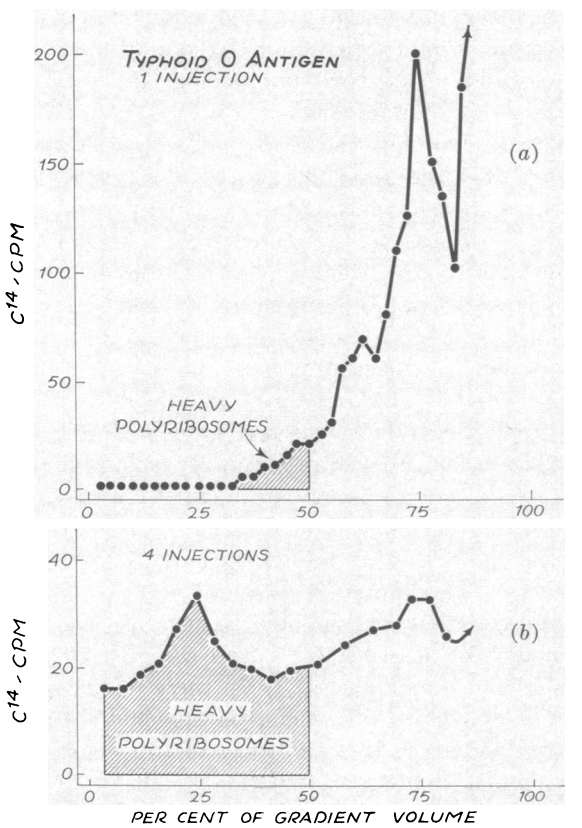

FIG. 1.—C^{14} amino acid incorporation into nascent protein of ribosomes from animals immunized with typhoid 0 antigen (*a*) one injection and (*b*) four injections. Single ribosomes in all gradients are located in the region of 75% of gradient volume. C^{14} amino acid incorporation in the lower 50% of gradients is arbitrarily labeled heavy polyribosomes.

To identify nascent gamma globulin, the gradients described above were collected in a total of 4 or 5 fractions, and each fraction divided into 2 parts. To one was added 0.1 vol of sheep anti-rabbit gamma globulin serum which was then brought to equivalence with rabbit gamma globulin in a concentration of 0.1 mg per ml. In the other, a similar preparation of rabbit antihorse gamma globulin and horse gamma globulin was precipitated at equivalence as a control. The precipitates which formed were centrifuged after standing overnight in the cold. The radioactivity of the supernatants and precipitates was counted.

The polyribosomes of the various fractions separated in the sucrose density gradients were also examined in the electron microscope.

The sera of the immunized rabbits which provided the lymphoid cells used for the isolation of the polyribosomes were examined for the presence of specific antibody, and identification of 19*S* and 7*S* antibody types was then carried out by sucrose gradient ultracentrifugation. On a 10–40% sucrose gradient (w/v) 0.5 ml of serum was layered and centrifuged for 15 hr at 25,000 *g*. Seven to ten fractions were collected. In the case of the typhoid 0 and sulfanilyl BSA-immunized animals, each fraction was tested for antibody activity either by typhoid 0 agglutination or by a capillary precipitation test with sulfanilyl BSA. Antibody activity in the lower $^1/_3$ of the gradient was taken as representing 19*S* antibody, while antibody activity in the upper $^1/_2$ was accepted as representing 7*S* antibody. Fractions were not examined for EA activity in rabbits immunized with this antigen since it was assumed that anti-EA antibody in such hyperimmunized animals was in the 7*S* form.

Results.—Figure 1*a* and *b* shows the results from animals given 1 and 4 injections of typhoid 0 antigen, respectively. In this and in all other gradients examined in the electron microscope, the fraction obtained at 75 per cent of the gradient volume

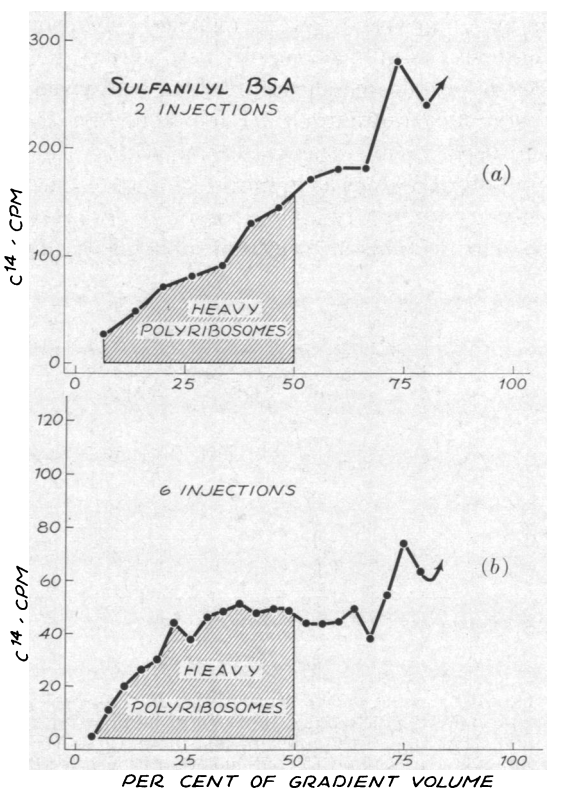

FIG. 2.—C^{14} amino acid incorporation into nascent protein or ribosomes from animals given (*a*) two injections, and (*b*) six injections of sulfanilyl BSA.

was made up of single ribosomes. Nascent protein-bearing ribosomes which migrate in the lower 50 per cent of the gradient have been arbitrarily labeled "heavy" polyribosomes. On electron microscopy, it was found that the polyribosomes sedimenting in the lower half of the gradients were composed of 5–10 ribosomes.

Animals which had not been immunized, or which had been last stimulated 4–6 weeks previously, showed almost all incorporation at the 75 per cent level, or in the region of single ribosomes. Thus, an animal given a single injection of typhoid 0 antigen (Fig. 1*a*) continued to demonstrate a majority of incorporation on single ribosomes. Significant incorporation was also present between 55 and 70 per cent of the gradients, but minimal incorporation was seen in the lower $^1/_2$ of the gradient. After 4 injections of typhoid 0 antigen (Fig. 1*b*), in an animal whose serum contained exclusively 19*S* antityphoid 0 antibody, a majority of incorporation appears to have occurred on the heavy polyribosomes migrating in the lower 50% of the gradient.

Similar results were obtained in animals immunized to produce 7*S* antibody, using sulfanilyl BSA as the antigen. Following 2 injections of this antigen (Fig. 2*a*), the most marked incorporation occurred in the single ribosome (75%) area, although significant incorporation was also present on the heavy polyribosome fraction. Following 6 injections (Fig. 2*b*), the major proportion of incorporation into nascent protein was found in the heavy polyribosomal area. At this time, the serum contained exclusively 7*S* anti-sulfanilyl BSA antibody.

To illustrate the apparent trend toward increased incorporation on heavy polyribosomes with progressive immunization observed above, the per cent of incorporation into total nascent protein which occurred on heavy polyribosomes is plotted against number of injections in Figure 3. Each point represents a separate animal. For each antigen utilized, an increasing fraction of C^{14} amino acid incorporation took place on heavy polyribosomes as immunization proceeded.

Since antigenically stimulated lymphoid tissue is undergoing a relatively rapid rate of cell division and contains a variety of morphologically different cell types,

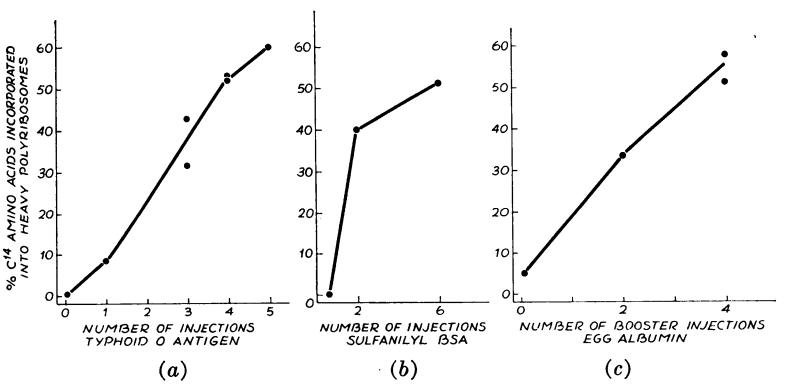

FIG. 3.—Per cent of total C^{14} amino acid incorporation into nascent protein which was associated with heavy polyribosomes is plotted as a function of numbers of injections of (*a*) typhoid 0 antigen and (*b*) sulfanilyl BSA; and (*c*) number of booster injections of hen egg albumin. Each point represents one animal. In (*a*) and (*b*), the incorporation after 0 injections refers to data from nonimmunized animals. In (*c*), the animals had received a course of primary immunization six weeks previously. In (*a*), the serum of the animal which received five injections of typhoid 0 antigen contained both 7*S* and 19*S* antityphoid 0 antibody.

TABLE 1

IDENTIFICATION OF NASCENT PROTEIN OF POLYRIBOSOMES OF LYMPHOID CELLS FOLLOWING IMMUNIZATION WITH SULFANILYL BSA*

Gradient volume (%)	Counts precipitated with rabbit γ globulin: antirabbit γ globulin (1)	Counts precipitated with horse γ globulin: antihorse γ globulin (2)	Specific counts precipitated (1–2)	Total counts in gradient fraction	Total counts specifically precipitated (%)
0–20	60	50	10	250	5
20–40	260	35	215	540	40
40–60	350	55	295	600	50
60–80	330	220	110	1300	9
80–100	960	840	120	6500	2

* Radioactivity is expressed as counts per 10 min.

it may be presumed that a wide variety of proteins are being synthesized. Hence, inferences relating polyribosomal populations solely to gamma globulin synthesis are not completely justified. For this reason, an attempt was made, using specific antigen-antibody precipitates, to identify antigenic determinants of rabbit gamma globulin associated with the polyribosomes. The animal used in this experiment had received six injections of sulfanilyl BSA. It is seen (Table 1) that antigenic determinants of rabbit gamma globulin were present in the nascent protein of an animal immunized with sulfanilyl BSA. These were noted in the portion of the gradient between 20 and 60 per cent of gradient volume. Relatively little active material was present in the area containing single ribosomes, i.e., between 60 and 80 per cent. Comparing these results with Figure 2*b*, it is seen that the antigenic determinants of gamma globulin coincide well with the principal distribution of polyribosomes from an animal immunized in the same way.

Discussion.—In the data reported, there appeared to be a relationship between the size of synthetically active polyribosomes which were isolated and the intensity of immunization. For all antigens used, a greater proportion of the polyribosomes from animals receiving the more prolonged courses of immunization migrated in the lower half of the gradients. This progressive increase in the size of active polyribosomes with increasing immunization appeared both with 19*S* antibody production following immunization with typhoid O and 7*S* antibody formation following immunization with egg albumin and sulfanilyl BSA. These results indicate either an enrichment of the lymph nodes by a distinct cell population containing larger polyribosomes, presumably plasma cells, or a progressive increase in polyribosomal size within the existing population. An alternative but less likely explanation for the observed increase in size is that increased breakdown of polyribosomes occurs in extracts of cells from less highly immunized animals, related possibly to a relatively higher concentration of macrophage derived degradative enzymes in those extracts.

The observation that the size of ribosomal aggregates increases during the course of immunization suggests a changing balance in the available amounts of messenger RNA and ribosomal RNA. The data support the possibility that, early in the course of antibody induction, messenger RNA would be available to bind the ribosomes present, leading thereby to the formation of the relatively small aggregates observed. If, later in the course of immunization, the ratio of messenger RNA to ribosomal RNA were reduced, the number of ribosomes associated with the messenger would increase. It seems likely that the observed shift in synthesis to heav-

ier polyribosomes after more intense immunization may account for the differing results which have previously been reported.[8-10]

The polyribosomal population which was found to be associated with antigenic determinants of gamma globulin and which was active in C^{14} amino acid incorporation was composed of aggregates containing 5–10 ribosomes. This indicates the presence of a messenger RNA large enough to code for a polypeptide of the range of 20,000–40,000 m.w.[4] This is the proper order of magnitude to account for the synthesis of H and L chains, which have molecular weights of 60,000 and 20,000, respectively, but is not of sufficient size to account for the synthesis of the total gamma globulin molecules.

The fact that the polyribosomes of 19*S* and 7*S* antibodies are similar in size argues against the presence of polycistronic messenger RNA for immunoglobulin synthesis. It is not possible to rule out with certainty, however, the possibility that larger, polycistronic messengers are present but not detected in these experiments. This could result from an incomplete "filling" of the messenger RNA with ribosomes, or from the breakdown of larger polyribosomes through the action of degradative enzymes. Although the upper limit of messenger RNA length cannot, therefore, be specified, it appears likely from the evidence presented that it is similar for both 7*S* and 19*S* gamma globulin.

The results obtained in these experiments are applicable to events occurring during normal immunization, whether the predominant specific antibody synthesized is of the 19*S* or 7*S* type. It is possible that nonspecific stimulation of immunoglobulin synthesis may occur, or that other antibodies not detected by current methods may have been produced during the course of immunization. In interpreting the data, however, it has been assumed that the observations made are related to the synthesis of the type of specific antibody detected.

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[1] Cohen, S., *Nature*, **197**, 253 (1963).

[2] Bauer, D. C., M. J. Mathies, and A. B. Stativsky, *J. Exptl. Med.*, **117**, 889 (1963).

[3] Finkelstein, M. S., and J. W. Uhr, *Science*, **146**, 67 (1964).

[4] Warner, J. R., P. M. Knopf, and A. Rich, these PROCEEDINGS, **49**, 122 (1963).

[5] Webb, T. E., G. Blobel, and V. R. Potter, *Cancer Res.*, **24**, 1229 (1964).

[6] Wettstein, F. O., T. Staehelin, and H. Noll, *Nature*, **197**, 430 (1963).

[7] Kretsinger, R. M., G. Manner, B. Gould, and A. Rich, *Nature*, **202**, 438 (1964).

[8] Stenzel, H. K., W. D. Phillips, D. D. Thompson, and A. L. Rubin, these PROCEEDINGS, **51**, 636 (1964).

[9] Manner, G., and B. S. Gould, *Nature*, **205**, 670 (1965).

[10] Scharff, M. D., and J. W. Uhr, *Science*, **148**, 646 (1965).

[11] Frenkel, E. P., B. E. Whalley, C. T. Knorpp, and D. R. Korst, *J. Lab. Clin. Med.*, **59**, 174 (1962).