as throughout the cytoplasm (Fig. 5). The ferritin molecules are engulfed by macrophages as evidenced by a large number of vesicles containing numerous granules along the surface of the cells. While the relative number of ferritin molecules taken up by the macrophages is far greater than that observed in the pinocytic vesicles of the lymphocytes, a single lymphocyte contains as many as several dozen molecules at a given plane of section, estimated to be 500 Å in thickness.

The use of ferritin molecules in tracing the fate of antigen by electron microscopy must be interpreted with caution since many, if not all, of the macrophages contain a large number of ferritin molecules within their cytoplasm. However, under the conditions of this study no such molecules were observed in lymphocytes from normal rats. This finding supports the contention that the molecules observed in the lymphocyte minutes after injection were antigenic ferritin. The meaning of this observation in terms of the role of the lymphocyte in antibody synthesis remains to be elucidated. Nevertheless, it is significant that antigenic ferritin molecules are taken up within a very short time after injection by small lymphocytes which are to be mobilized shortly afterwards (*6*). These small lymphocytes can be transformed into large blast cells by antigen (*7*). Whether or not the uptake of ferritin by small lymphocytes represents specific recognition of ferritin molecules as antigen awaits a comparative study of the fates of isologous and heterologous ferritin molecules.

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RNA Composition and Base Pairing

Abstract. *If RNA may contain a small proportion of adenine-guanine base pairs, these could interrupt the continuity of helical structure in a polynucleotide, in keeping with current theories of RNA structure, and could also account for the experimentally observed tendency for 6-amino bases to equal 6-keto bases and for purines to exceed pyrimidines.*

The striking regularities in the nucleotide composition of DNA (*1*) stem directly from the structure of the molecule, in which A (*2*) is paired with T and G with C (*3*). The regularities—A = T, G = C, A + G = C + T (Pu = Py), and A + C = G + T (6 Am = 6 K)—are imposed by this structural feature.

It has been pointed out that the nucleotide composition of the total RNA from various sources, reflecting mainly the composition of the ribosomal RNA, tends to show one of these regularities, 6 Am = 6 K, but not the others (*4*). Tentative explanations were proposed in terms of structure (*4*) and, later, of information theory (*5*). Since then evidence has accumulated which indicates that both the ribosomal and transfer RNA's possess a relatively high, though incomplete, degree of base pairing of the DNA type, with U replacing T (*6, 7*). Furthermore, x-ray studies of two viral RNA's (*8, 9*) and of fragments of ribosomal RNA (*10, 11*) have shown

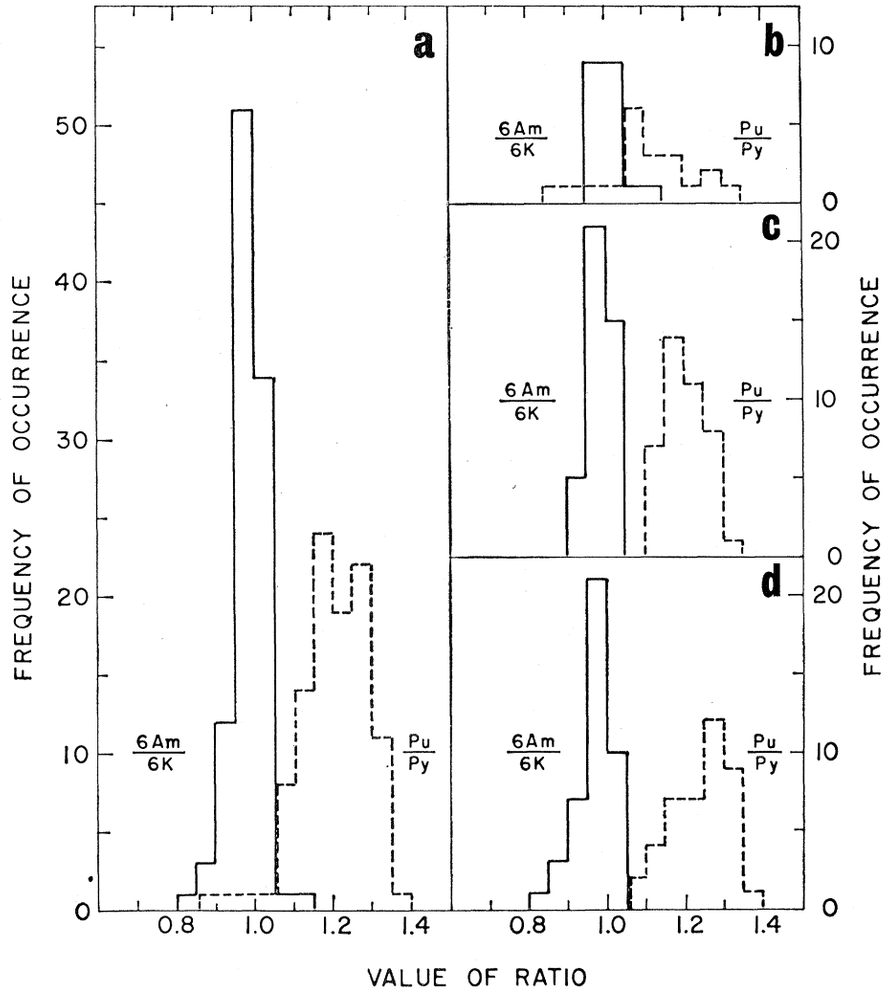

Fig. 1. Frequency distributions of the compositional ratios 6 Am/6 K and Pu/Py of total RNA from 103 different species. The data were taken from references *4* and *12*; where more than one value was listed for a single species, the average value is plotted. *a*, The total data (103 species), which were then divided into three groups as follows: *b*, animals (20 species); *c*, algae and higher plants (41 species); *d*, bacteria and fungi (42 species). Mean values of 6 Am/6 K for groups *a, b, c,* and *d* are 0.98, 1.00, 0.98, and 0.96, respectively. Mean values of Pu/Py for groups *a, b, c,* and *d* are 1.19, 1.11, 1.19, and 1.23 respectively.

that they form double-stranded helical structures with dimensions similar to those of DNA, again suggesting that the base pairs of DNA also occur in RNA. In itself, this might tend to produce a general bias in favor of the relationship 6 Am = 6 K, but the other compositional regularities found in DNA should, then, appear in RNA to an equal degree, and this is not the case.

After the earlier survey, analyses of total RNA from many phylogenetically diverse species were compiled, and the data provide strong support for the generalization that 6 Am = 6 K (*12*). It is apparent from these data that, in general, total purines exceed total pyrimidines. When the data from the three compilations of total RNA composition (*4, 12*) are tabulated (Fig. 1) the values for 6 Am/6 K are closely grouped about 1.0 (*13*). The values for Pu/Py are more broadly distributed but, with very few exceptions, fall between 1.0 and 1.4. We suggest that if, in addition to the standard G-C and A-U pairs, base pairing can also occur between A and G, the features of the composition noted above can be accounted for, not as invariable rules, but as general tendencies.

An A-G base pair with the hydrogen bonds shown (Fig. 2) has been postulated (*15*). In this pair the adenine moiety is rotated 180° about its bond to the sugar from the standard orientation. Models indicate that this is quite feasible for adenine, though probably not for the pyrimidine nucleotides, where there is severe steric hindrance to such a rotation. Of the 29 ways of forming hydrogen-bonded pairs with the four bases of DNA examined (*15, 16*), this A-G pair is the only one in which the separation and orientation of the nitrogen (base) to the carbon (sugar) bonds are closely similar to those of the G-C and A-T pairs of DNA (*17*); and it was suggested that it might be inserted, in either order, into the Watson-Crick structure for DNA with only very small distortions (*15*).

Our models show, however, that this is not possible in the fully ordered structure of DNA, owing to short contacts between the carbon No. 2 and nitrogen No. 3 atoms of adenine and the carbon No. 2′ sugar atom of the adjacent nucleotide attached to the 5′ position of the adenylic acid residue. It is possible that in double-helical regions of RNA the corresponding adenine and sugar residues are sufficiently far apart to avoid these close contacts; x-ray

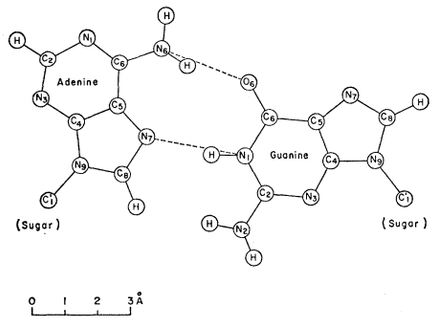

Fig. 2. Suggested pairing of adenine and guanine (*15*).

structure analyses of RNA, precise enough to settle this point, have not yet been reported. However, it appears more likely that if such an A-G pair were formed, it would only occur next to an appropriate break in the base-paired sequence so that the neighboring sugar in question would be moved out of position. If the break were at the end of a double-helical region, it would also enable the 2-amino group of guanine, when rotated somewhat out of the plane of the base pair, to be fully hydrogen-bonded to water (*18*). Because of the equivalent positions of the glycosidic bonds in the A-G pair and the two standard base pairs, there would be no appreciable distortion at the end of the helix, and intrachain hydrogen bonding could be maintained between the 2′-ribose hydroxyl and the ring-oxygen atom of the neighboring ribose or an oxygen of the adjacent phosphate group (*8, 10*). In short, this A-G pair could be fitted into a double helix, with the adenine at the 5′ end but not at the 3′ end of the helical region of a polynucleotide chain, and it would cause the termination of the helix at that point.

Such an A-G pair can be fitted nicely into the model for RNA proposed by Fresco *et al.* (*19*). According to this model RNA contains many short DNA-like helical regions which are formed when local stretches of the single polynucleotide chain fold back upon themselves. These regions are stabilized by A-U and G-C pairing and have been estimated to constitute the major portion of the molecule (*6*). Unpaired nucleotides are found in the hairpin turn at the end of the helix, in the stretches connecting the helical regions, and perhaps in small loops which are thrown out from the helical segments in order to allow an adjustment towards more perfect complementarity (*19*). Support for some aspects of this postulate has been provided by the crystallization of ribosomal fragments which x-ray diffraction studies show have a double-helical structure (*10, 11*). A relatively small amount of A-G pairing could provide a structural basis for the termination of many such helical regions within a single polynucleotide chain.

Such A-G pairing could also account for the observed compositional features. Fig. 1*a* shows that the ratios of 6 Am to 6 K of 103 species lie almost entirely between 0.90 and 1.05 with a mean of 1.0, while those of Pu to Py are mainly grouped between 1.05 and 1.35 with a mean value of 1.2. As an illustrative example we may fit a hypothetical RNA segment to these numbers. A fully base-paired stretch of ten standard pairs plus one A-G pair would have a ratio of 6 Am to 6 K of 1.0 and a ratio of Pu to Py of 1.2. If there were either more or fewer standard pairs in the segment, the ratio of 6 Am to 6 K would remain unchanged but the ratio Pu to Py would change. This might account for the greater variability of the ratio of Pu to Py, which has been observed experimentally. If two variable unpaired nucleotides were added to the 22 paired nucleotides the ratios of 6 Am to 6 K would be between 0.85 and 1.18 and of Pu to Py between 1.0 and 1.4, in rough agreement with the observed variation. In sum, helical regions terminated by an A-G pair would confer on the composition of RNA a tendency toward the relationships 6 Am = 6 K and Pu > Py, with Pu/Py varying inversely with the average length of the helical segments. The non-helical regions, insofar as their composition differs from the ratios of the helical regions, would cause deviations from these values. If the helical content were high, as is believed, then there would be a marked tendency towards the relationships which have been observed.

There are indications of a greater similarity in nucleotide composition among closely related species than among those from more distantly related biological groups (*4, 12, 14*). Fig. 1, *b–d*, shows that, whereas the distributions of 6 Am/6 K values for all three groups are quite similar, the Pu/Py values for animals are generally appreciably lower than for the other groups. Similarly, for a small sample of bacteria, ratios of Pu to Py appear higher for 50*S* than for 30*S* ribosomes (*14*). Such a variation in composition might stem not only from differences in

the composition of nonhelical regions but also from differences in the relative amount of helical structure and in the lengths of the helical segments. Variation in composition might, therefore, reflect significant differences in structure.

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Beta-1C-Globulin: Metabolism in Glomerulonephritis

Abstract. *The metabolism of β_{1C}-globulin labeled with iodine-131 was studied in six normal individuals and in three individuals with glomerulonephritis who exhibited markedly reduced serum concentrations of this protein. Fractional catabolic rates were similar in both groups, and therefore the low concentration of serum β_{1C}-globulin in glomerulonephritis appears to be chiefly secondary to decreased synthesis.*

It has been known for decades (*1*) that total serum complement activity is markedly lowered for several weeks after the onset of acute glomerulonephritis. In addition, some children without a history of acute glomerulonephritis, but with intermittent nephrotic syndrome and characteristic pathological changes of the glomeruli (progressive glomerulonephritis), have persistently low levels of complement activity for a year or more (*2*). In both acute and progressive glomerulonephritis, concentration of the third component of complement, β_{1C}-globulin (C′3), has been shown to be markedly decreased in serum (*2, 3*).

Several independent observations have indicated that the lowered activity of complement in these disorders is the result of continuing complement fixation. Immunofluorescent studies have shown β_{1C}-globulin, as well as γ-globulin and fibrinogen, on the glomeruli of patients with glomerulonephritis (*4*). Some features of acute and progressive glomerulonephritis can be produced in rats by the injection of rabbit antiserum to rat kidney (*5*). In this experimental disease, activity of serum complement is lowered and β_{1C}-globulin is fixed to glomeruli (*5*).

We have attempted to obtain more direct evidence for the basis of persistently lowered activity of complement in the serums of individuals with glomerulonephritis by the use of I^{131}-labeled β_{1C}-globulin. $Beta_{1C}$-globulin was prepared by the original method of Müller-Eberhard, Nilsson, and Aronsson (*6*), and it was labeled with radioactive iodine by the technique of MacFarlane (*7*). A mean of one atom of iodine (or less) per molecule was incorporated. Over 80 percent of the radioactivity was attributable to β_{1C}-globulin as measured by precipitation of counts in antibody excess by an antiserum specific for this protein.

The labeled β_{1C}-globulin was filtered on a Millipore filter and examined by immunoelectrophoresis before and after incubation with hydrazine and $EAC'_{1,4,2}$ (sheep red blood cells sensitized with rabbit antibody to sheep red cells that have complement components 1, 4, and 2 on them), and, after addition to fresh serum, by radioimmunoelectrophoresis, with the use of antiserums to whole human serum and to β_{1C}-globulin. With both antiserums, the labeled protein gave an arc identical in appearance with that of native β_{1C}-globulin in fresh serum. The same material converted to an arc of more rapid mobility (β_{1A}- or β_{1G}-globulin) when it was incubated with hydrazine or $EAC'_{1,4,2}$ in the same manner as β_{1C}-globulin in whole serum. Millipore-filtered, labeled, and dialyzed β_{1C}-globulin increased the hemolytic activity of serum from patient V.O. (with progressive glomerulonephritis) to the same extent that the unlabeled protein did. These findings confirm the observation of Müller-Eberhard and coworkers that labeling with radioactive iodine has no effect on the complement activity of C′3 (*8*).

Concentration of serum β_{1C}-globulin was estimated by an immunochemical method (*9*). Six normal subjects and three individuals with glomerulonephritis were studied (*10*). After intravenous injection of from 2 to 6 μc of I^{131}-labeled β_{1C}-globulin, radioactivity was assayed in plasma, urine, and, in some cases, stool. In no case was significant protein-bound radioactivity found in the urine, nor was there significant excretion of radioactivity in the stool. Curves of protein-bound radioactivity in plasma and urinary excretion in normal individuals (Fig. 1) and in individuals with glomerulonephritis (Fig. 2) showed no striking differences. Since the concentrations of β_{1C}-globulin in serums of individuals with glomerulonephritis were between 4 and 20 percent of normal (5 to 30 mg as opposed to a normal of 150 ± 10 mg per 100 ml), the low levels resulted primarily from depressed synthesis of β_{1C}-globulin.

Catabolic and synthetic rates were calculated from curves of protein-bound radioactivity in plasma by the Matthews method (*11*) and from plasma concentrations of β_{1C}-globulin. Plasma volumes were calculated from the amount of radioactivity in plasma samples obtained 10 minutes after injection and the amount of radioactivity in the labeled protein that was injected. In several instances, plasma volumes were also determined by I^{125}-labeled human serum albumin, and the figures