mice with lymphoma were being treated with asparaginase, a concentration of 7 or more units per milliliter was maintained in the blood plasma for 24 hours after one injection of 15 units per gram of body weight.

Lymphocytes from 12 of the 15 patients showed a higher sensitivity to 10 and 100 units of L-asparaginase per milliliter than lymphocytes from any of the 25 control persons (Fig. 1). The sensitivity of lymphocytes from only two patients (SM and KC) was within the range for normal lymphocytes. Repeat tests were done on cells of six patients. Lymphocytes from one of them (PM) had a relatively low sensitivity to 10 and 100 units on the first test (Fig. 1) and in two repeat tests. Another patient (MU) had a high sensitivity to both 3 units and 1 unit on the first test; a repeat test showed a high sensitivity to 3 units but not to 1 unit. In all six patients, the findings on one or two repeat tests were consistent with the findings obtained in the first test.

Human leukemic lymphocytes were found to have a relatively high sensitivity to guinea pig serum in a previous study (*9*) and to a purified preparation of L-asparaginase from *E. coli* in this one. The only known common factor in the two reagents is L-asparaginase and presumably this enzyme has a greater toxicity for leukemic than for normal lymphocytes in vitro.

The difference in sensitivity of normal and leukemic human lymphocytes to L-asparaginase raises the question of the asparagine requirements of cells. The amino acid is not required for in vitro growth of several types of normal and malignant cells of man (*16*) and lower animals (*17*) but is required by cells of Walker rat carcinosarcoma 256 (*18*) and of mouse leukemia L5178Y (*19*). L-Asparaginase is required in the culture medium by cells of mouse lymphoma 6C3HED (*20*), a tumor which regresses in vivo on treatment with guinea pig serum or with L-asparaginase. In contrast, L-asparagine is not necessary for the continuous cultivation of a derived lymphoma 1RG which is resistant to in vivo treatment by guinea pig serum (*20*). These studies indicate that asparagine is not required for culturing many types of normal and tumor cells but is essential for growth of cells of two mouse tumors and one rat tumor. Preliminary work (*21*) showed that C^{14}-asparagine was incorporated by lymphocytes from seven patients with chronic lymphocytic leukemia and from four control persons. The requirements of normal and leukemic human lymphocytes for L-asparagine are not known.

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Mammalian Ribosomal Protein: Analysis by Electrophoresis on Polyacrylamide Gel

Abstract. *Ribosomal protein from five mammalian tissues when analyzed by discontinuous electrophoresis on polyacrylamide gel at* pH *4.5 yielded 24 bands. Densitometric tracings indicated that the patterns of the basic ribosomal proteins from the several tissues were qualitatively similar. Protein from* Escherichia coli *ribosomes analyzed at* pH *4.5 gave 29 bands, and the pattern was different from that of mammalian ribosomal protein. No distinct band was found when mammalian ribosomal protein was analyzed at* pH *8.3 (acidic proteins). Ribosomal protein from* Escherichia coli *gave eight bands at* pH *8.3. Thus, the structure of the genes responsible for synthesis of ribosomal protein in several mammalian tissues is the same, and different genes direct synthesis of ribosomal protein in bacteria.*

Whether the cistrons for ribosomal protein are the same in different mammalian tissues has not yet been determined. Although a substantial amount is known about the electrophoretic characteristics of bacterial ribosomal protein (*1–4*), only one report (*5*) of analysis of ribosomal protein from mammalian tissues has appeared.

Ribonucleoprotein particles were isolated from rat liver (*6*), kidney (*6*), skeletal muscle (*7*), and cardiac muscle (*8*); from rabbit reticulocytes (*9*); and from *Escherichia coli* (*3*). The ribosomes were washed with suitable media until the absorbancy ratios, 260/280 mμ and 260/235 mμ, were greater than 1.75 and 1.50. Portions of the ribosomes from skeletal and cardiac muscle, from reticulocytes, and from *E. coli* were assayed (*10*) and found capable of catalyzing the synthesis of protein.

Protein extracted from the ribosomes with 8*M* urea and 4*M* LiCl was subjected to electrophoresis on polyacrylamide gel (*3*). The urea-lithium chloride method for the extraction of ribosomal protein is more reproducible than other methods, provides better separation of the protein from RNA, and gives good recovery (*4*); the protein prepared by this method yields on electrophoresis the largest number of bands (*11*). Usually the concentration of the separation gel was 10 percent, but for the resolution of the mammalian ribosomal protein bands 12 to 14 (compare Fig. 1), it was reduced to 7.5 percent. At the lower concentration the number of bands resolved is greater but many individual bands are less sharply defined. A 10-μl sample containing 50 μg of ribosomal protein was placed on the gel with 1 μl of tracker dye—0.1 percent pyronin Y for runs made at *p*H 4.5, and 0.1 percent bromphenol

blue for those at *p*H 8.3. Care was taken that the quantity and especially the volume of protein were the same for each analysis. Electrophoresis was conducted at 3°C and 3 milliamperes for 150 minutes at *p*H 4.5 (migration toward the cathode) or for 90 minutes at *p*H 8.3 (migration toward the anode). The gels were stained with 1 percent amido black in 7.5 percent acetic acid, and the excess stain was removed by electrophoresis overnight. The gels were then scanned with white light with a Canalco model F microdensitometer.

Ribosomal protein from mammalian tissues (rat liver, kidney, skeletal and cardiac muscle, and rabbit reticulocytes) gave 24 bands when electrophoresis was carried out at *p*H 4.5. The patterns (Fig. 1) of the mammalian ribosomes exhibit great similarities, although they are not exactly congruent; for example, the peak complexes numbered 8 to 10 and 15 to 18 vary. There are several possible reasons for the variation. The quantity of one or more of the proteins may differ; inspection of the gels and examination of the densitometric tracings suggest this possibility, but we have not yet directly determined the relative amounts of the 24 proteins. Gel preparations may vary in composition and degree of cross-linking and hence alter the conformation of the banding pattern. Variations in background staining, in the current, or in the temperature during electrophoresis may also change the pattern. Given the large number of variables, such good correspondence as there is among all the bands is perhaps surprising. The best guarantee for rigid control of each variable, so necessary for precise comparison of particular bands in separate preparations, is the use of the "split-gel" technique (*3*).

Two different samples of ribosomal protein were placed on a single gel column, but separated by a liquid-tight insert that split the upper two gel layers longitudinally into two compartments. Electrophoresis was then carried out in the usual manner (Fig. 2). The correspondences of the bands confirm the indication of the densitometric tracings that the ribosomal proteins from the four mammalian tissues are qualitatively identical.

The pattern obtained on electrophoresis of mammalian ribosomal protein can be readily reproduced. We have analyzed as many as six separate preparations from a single tissue and have

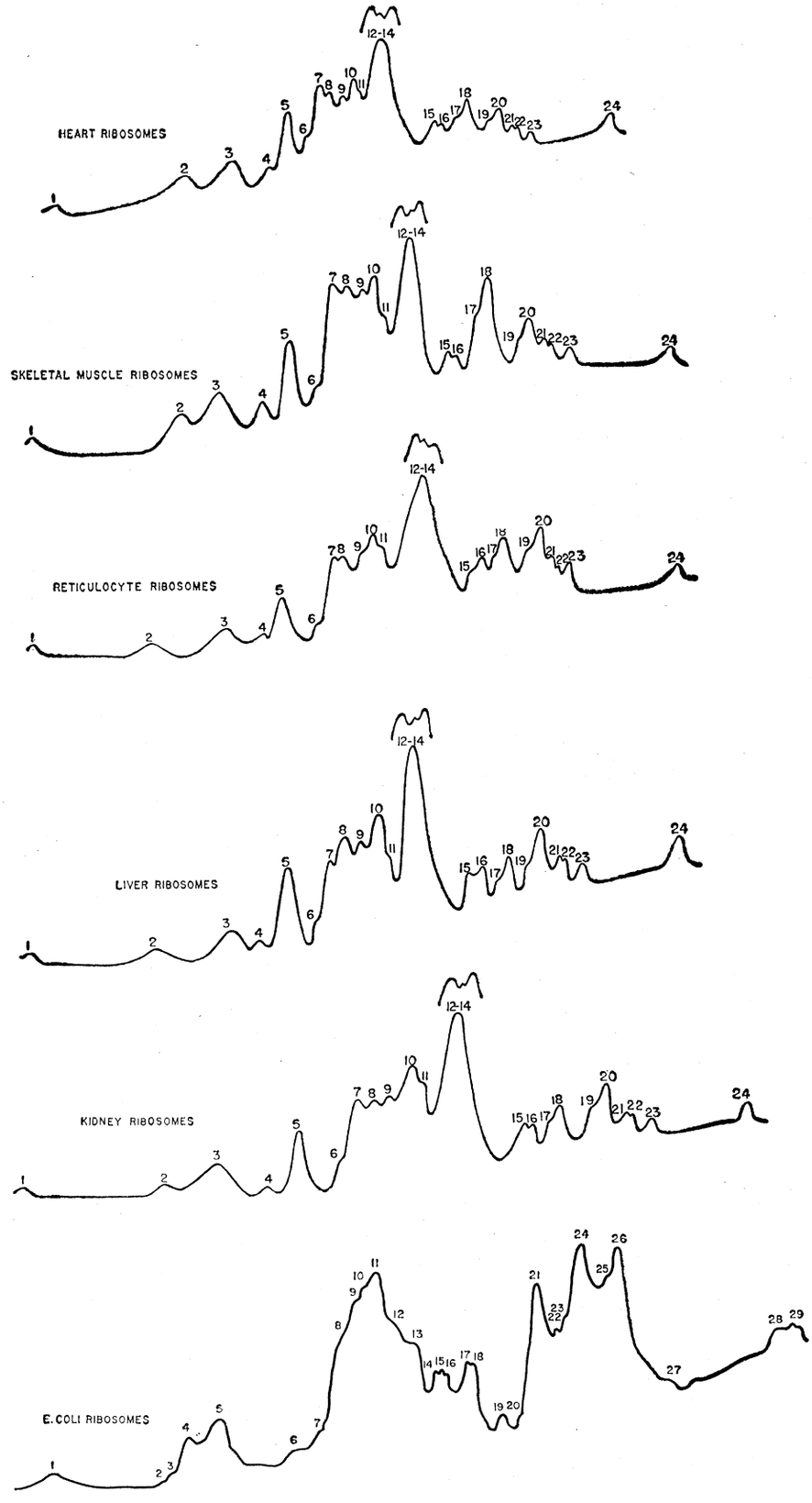

Fig. 1. Densitometric tracings of polyacrylamide gels after discontinuous electrophoresis of ribosomal protein from several mammalian tissues and from *E. coli*. Electrophoresis was from the right (anode) to the left (cathode). The concentration of the separation gel was reduced from 10 to 7.5 percent to resolve bands 12 to 14 (see insets). The numbers assigned to the bands are for comparison of ribosomal protein from the five different mammalian tissues; the numbers assigned to the *Escherichia coli* ribosomal protein bands are only for convenience; they do not imply a homology with the mammalian ribosomal protein bands having the same number. The distance of migration of the proteins was not exactly the same for each preparation.

observed exact correspondence of the bands; that has been true also when we have run a single preparation in triplicate.

Waller (*2*), using ion-exchange chromatography and starch-gel electrophoresis to analyze ribosomal protein from *E. coli*, was able to detect at least 24 bands, the majority being basic proteins. Fractionation by means of polyacrylamide-gel electrophoresis has confirmed the heterogeneity of bacterial ribosomal protein (*3*). The majority of proteins—24 to 30—are basic, but several—6 to 9—are acidic (*11*). We found that protein from *E. coli* ribosomes showed, on electrophoresis at *p*H 4.5, 29 bands and a pattern different from that of mammalian ribosomal protein (Fig. 1). We do not know whether all or only some of the ribosomal proteins from *E. coli* are different from those of mammalian tissues.

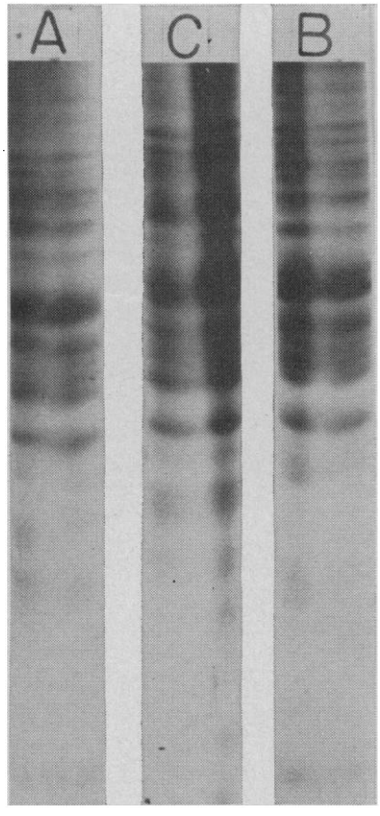

Fig. 2. Electrophoretic patterns of basic ribosomal proteins analyzed on polyacrylamide gel by the "split-gel" technique. The comparison in A is between protein from liver ribosomes (on left) and heart muscle ribosomes (on right); in B it is between protein from liver ribosomes (on left) and skeletal muscle ribosomes (on right); and in C, between protein from liver ribosomes (on left) and reticulocyte ribosomes (on right). Electrophoresis was from the top (anode) to the bottom (cathode). The concentration of separation gel was 10 percent.

No acidic protein could be consistently identified in the preparation of mammalian ribosomal protein. Occasionally one to four bands appeared when electrophoresis was carried out at *p*H 8.3, but large amounts of protein (five times the quantity ordinarily used) were required, and even then the bands were not reproducible from preparation to preparation. We cannot, therefore, be certain whether or not mammalian ribosomes contain acidic proteins. In contrast, preparations from *E. coli* always gave seven or eight acidic proteins.

There is no certainty concerning the exact number of distinct and separate proteins present in the mammalian ribosome. Possibly the preparation or the electrophoresis of the ribosomal proteins leads to breakdown of one or a few proteins to give 24 bands. The reproducibility of the electrophoretic pattern seems to indicate that this is not the case. More likely, some minor components were not resolved, and mammalian ribosomes actually contain more than 24 basic proteins. Whether electrophoresis is a valid technique for resolving heterogeneity can be confirmed only when several of the protein bands have been isolated and purified, and when their amino acid composition and sequence is determined (*3*). But whether there are few or many mammalian ribosomal proteins, they give qualitatively similar electrophoretic patterns; therefore, they must at least be derived from the same parental proteins.

There are reservations to the conclusions derived from such complicated protein patterns. At best we should only be able to detect the deletion or addition of a protein band, and not even that under all circumstances. For example, if a particular protein were present in the ribosomes of one tissue, but in an amount so small relative to the other proteins as not to be detectable, its deletion from the ribosomes of a second tissue could not be determined.

Moreover, all the minor protein components may not be resolved from the more prominent bands; the addition or deletion of such a minor band would also escape detection.

At least 24 distinct basic proteins can be resolved from mammalian ribosomes by electrophoresis on polyacrylamide gels. Since the patterns of the proteins are similar, it seems reasonable to conclude (subject, of course, to the reservations already discussed) that the same cistron or cistrons direct the synthesis of ribosomal protein in the several mammalian tissues and that one or more different cistrons direct the synthesis of bacterial ribosomal protein.

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Glucans of Oomycete Cell Walls

Abstract. *The cell walls of selected oomycetous fungi are composed primarily of glucans, and cellulose constitutes a relatively small proportion of the total glucan. The noncellulosic constituents consist of acid-soluble glucan or glucans and insoluble glucan or glucans. These noncellulosic glucan fractions contain β-(1→3) glucosidic linkages and apparently β-(1→6) linkages also.*

Cell walls of fungi are known for their chitinous nature which distinguishes them from the cellulosic walls of green plants (*1*). The Oomycetes (*2*), however, may be readily distinguished from the majority of fungi since their walls do contain cellulose, and appar-