sRNA; Phe, phenylalanine; Ser, serine; Leu, leucine; Ileu, isoleucine; Tyr, tyrosine; Cys, cysteine; Val, valine; Arg, arginine; Asp, aspartic acid; Glu, glutamic acid; His, histidine; Lys, lysine; Thr, threonine.

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THE MOLECULAR BASIS FOR THE GENETIC CODE*

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The central problem of the genetic code is to identify the factors giving rise to the observed codon assignments. In particular, the key issue is whether or not amino acid-oligonucleotide steric interactions play or have played a role in determining these assignments, and if so, to what extent.[1] Although in the beginning there were no pertinent facts bearing on this matter, there initially was certainly no dearth of theoretical speculation. Gamow and others assumed amino acid-nucleic acid steric interactions to be the sole factors determining codon assignments.[2]

Crick, on the other hand, vigorously challenged this amino acid-nucleic acid complementarity dogma, arguing that since neither evidence for such interactions nor a reasonable model for them existed, the interactions themselves did *not* exist under *any* circumstances. From this basic tenet Crick then derived the now famous adaptor hypothesis (and thence the comma-free codes).[3] Admitting no possibility of an oligonucleotide's "recognizing" an amino acid meant, of course, that the assigning of a codon to a particular amino acid would be a chance event—a matter of "historical accident"—making the reason for any particular assignment totally uninteresting.[4]

Practically all the codon assignments are now known, and they are seen to form a very highly ordered array.[5] This catalogue, as it is called, not only manifests constraints regarding the grouping of codons to individual amino acids, but also constraints defining the codons which are assigned to "related" amino acids.[6] The existence of such constraints is not, as might at first be thought, incompatible with the adaptor hypothesis' "historical accident" view of the genetic code.[7] Thus, even the knowing of the codon catalogue in its entirety does not permit one to distinguish between the diametrically opposed views regarding the basic reason for the assignments.

In an effort to decide the issue of whether or not these "recognition" interactions —henceforth to be called "codon-amino acid pairing"—are behind the codon assignments, we have been studying the possibility of interactions between amino acids and various organic bases. While it is still too early to say for certain that interactions between amino acids and oligonucleotides definitely played a role in determining codon assignments, the data presented herein certainly do suggest this possibility most strongly.

Materials and Methods.—The methods used herein are well-known standard paper chromatographic ones.[8] The amino acids were obtained from either Mann Laboratories or Calbiochem Laboratories; the solvents from either Aldrich Chemicals or Eastman Kodak. Whatman #2 paper was used throughout.

Results and Specific Discussion.—The existence and general nature of amino acid-organic base interactions: The first point to consider is whether nucleic acid-amino acid interactions or, more generally, organic base-amino acid interactions really exist. There have been in the past a number of attempts to detect the former (e.g., by equilibrium dialysis), but these have yielded negative results (except for the obvious charge-charge interactions involving lys or arg).[9] This negative evidence, however, is meaningless—except perhaps to suggest that one attempt to demonstrate such interactions under conditions where water is not ubiquitous. For this reason and for the reason that there appears to be some correlation between a ranking of amino acids by their codon assignments and a ranking by their chromatographic behavior, we have been particularly interested in the possibility of amino acid-pyridine interactions, as might be manifested in paper chromatography.

The simple chromatographic separations of amino acids in pyridine solvents do not themselves prove the existence of amino acid-pyridine interactions. The reason for this is that in order to move the amino acids, a pyridine solvent must contain some water, and merely by supposing the amino acids to travel in association with or surrounded by a characteristic number of water molecules, one could ostensibly account for their chromatographic behavior. A very strong argument that

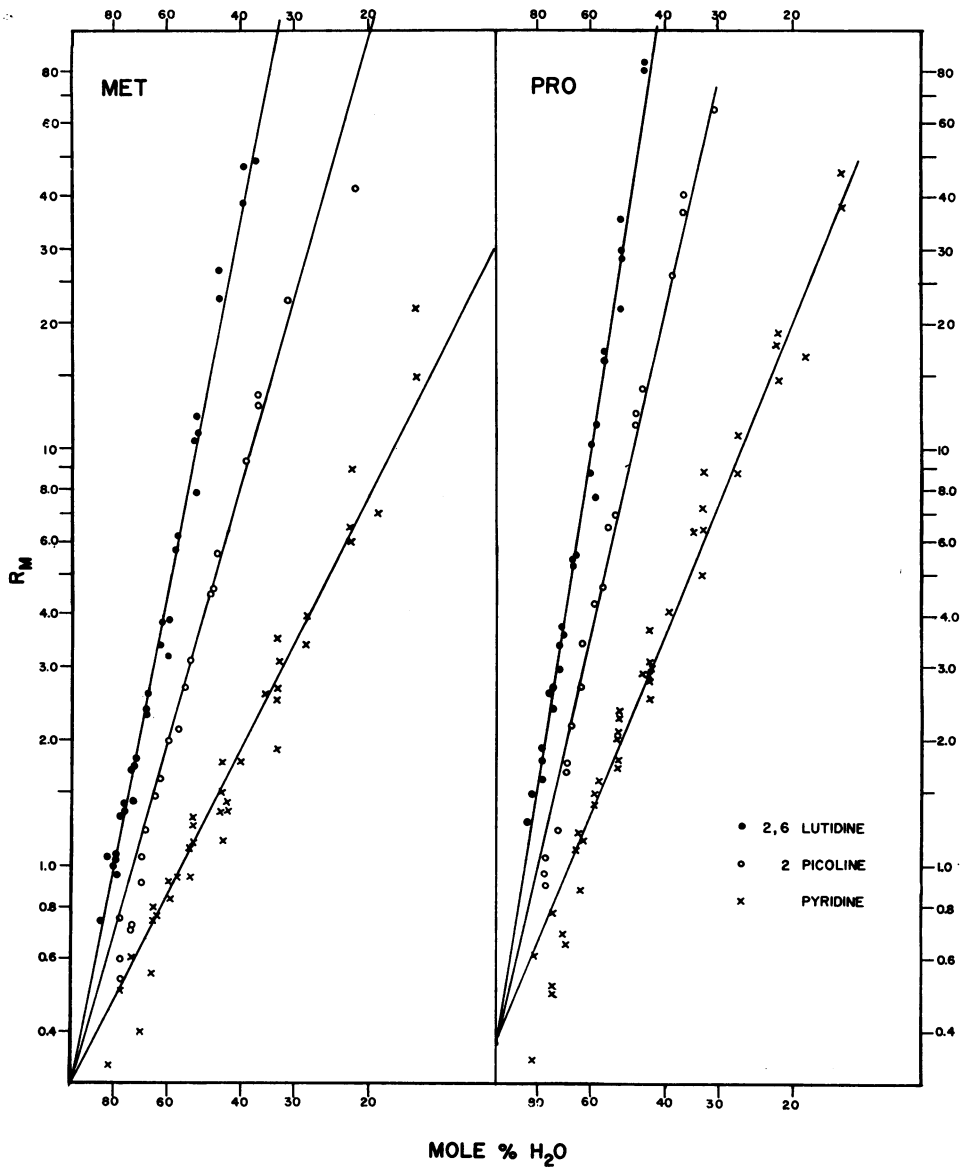

FIG. 1.—Log amino acid R_M vs. log mole fraction H_2O in the chromatography solvent.

pyridine and amino acids do indeed interact is the following: If one plots log amino acid R_M vs. log mole fraction water for a series of pyridine:water mixtures, one obtains a straight line.[10] (We arbitrarily define R_M here as $1/R_F - 1$.) Figure 1 shows examples of such plots. The most straightforward interpretation of this linear relationship is that in the solvent phase there are n molecules of water associated with the amino acid, the number n being determinable from the slope of the plot. It is interesting to compare such slopes for pyridine (P), 2-Me pyridine (MP), and 2,6-diMe pyridine (DMP) solvents. For the case of MP there is a par-

tial, and for DMP a greater, or even complete, blockade of the amino acid's access to the ring N. Accordingly, as Figure 1 shows, the dependence upon water, i.e., the number of water molecules apparently bound by the amino acid, is seen to increase dramatically for the "blocked" solvents. However, 3MP, 3,4 DMP, etc., behave almost as does P, as one might expect. These data are consistent with and tend to confirm our working hypothesis that the slopes of R_M-water plots reflect the binding of water by amino acids. The relatively low slopes in pyridine indicate also that pyridine is replacing *some* of the water in an amino acid's "coordination sphere," and so *can* indeed interact with the amino acids. Further, this pyridine-amino acid interaction, as is the case with water binding, must be a *polar* one, at least in part. The evidence (if such is really needed) for the essentially self-evident fact that pyridines additionally interact with amino acids in a *nonpolar* fashion will be presented elsewhere, as will a further characterization of the *polar* aspect of the interaction.[11]

In brief then, from the evidence presented here and elsewhere,[11] we conclude simply that organic base-amino acid interactions do exist and comprise (to a first approximation) two aspects—a *polar* interaction of ring N's, etc., on the bases with the polar portions of the amino acids, plus a *nonpolar* interaction, between the more "organic" portions of the bases and amino acids.

Characterization of the amino acids by polar requirement: The above R_M-water plot slopes turn out to be extremely important as a means for characterizing the amino acids and defining their relatedness. Why these slopes would tend to be different for different amino acids can be understood in terms of the above model for organic base-amino acid interactions. For an amino acid to exist in the solvent phase requires a certain energy of interaction between it and the solvent, and, of course, both the polar and the nonpolar aspects of its interaction contribute to this. Therefore, if the amino acid and solvent can interact relatively strongly in a nonpolar fashion, there will be relatively less of a need for polar interaction. Thus, we expect that certain amino acids—like leucine, for example, which has a rather large aliphatic R group—to have lower slopes to their R_M-water plots than do other amino acids—alanine, with a smaller aliphatic R group, for example. And this expectation is, of course, borne out. A comparison of the ordering of amino acids by the slope of their R_M-water plots, which we shall henceforth call "polar requirement,"

TABLE 1

	U_{II}		C_{II}		A_{II}		G_{II}		
	Phe	5.0			Tyr	5.7	Cys	11.5	U_{III} C_{III}
U_I			Ser	7.5					
	(Leu)				—		Try	5.3	A_{III} G_{III}
					His	8.4			
C_I	Leu	4.9	Pro	6.6			Arg	9.1	
					Gln	8.6			
	Ilu	4.9			Asn	10.0	(Ser)		
A_I			Thr	6.6					
	Met	5.3			Lys	10.1	(Arg)		
					Asp	13.0			
G_I	Val	5.6	Ala	7.0			Gly	7.9	
					Glu	12.5			

The polar requirements of the amino acids, which are ordered by codon assignment. (For example, an amino acid in the C row and the U column will have C in the I position of its codons, U in the II position, U, C, A, or G in the III position if it is the only amino acid in that category. If two amino acids share a category, the upper one has U or C in the III position of its codon, the lower, A or G. The minor occurrences of an amino are in parentheses.)

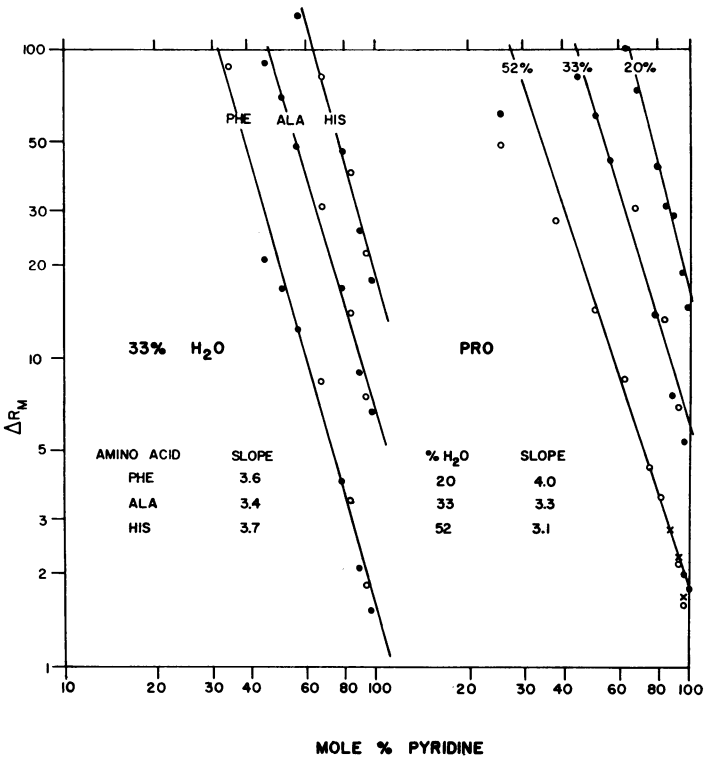

FIG. 2.—Log ΔR_M vs. log mole fraction pyridine. Amino acids are chromatographed in a series of pyridine, 2,6-diMe pyridine, water mixtures. The mole fraction of water is held constant throughout and the ratio of P to DMP varied. The slope of plots of log ΔR_M vs. log mole fraction P measures the number of pyridine molecules bound to an amino acid (see text for details).

to their ordering by codon assignment is most informative. Table 1 shows such a ranking. (These numbers hold for 2,6 DMP solvents, but essentially identical relative orderings are obtained when P or MP solvents are employed—except, of course, the absolute values of polar requirements are less in these latter cases.) The obvious features of Table 1 to note are: (1) amino acids, all of whose codons differ *only* in the III position, are almost identical in their polar requirements—ilu-met, his-gln, asn-lys, asp-glu are the cases in point, and cys-try appears to be an exception;[12] (2) all amino acids having codons with U in the II position (U_{II} codons) have very nearly the same polar requirement, which is likewise the case for amino acids having C_{II} codons. There thus appears to be a striking correspondence between a polar requirement ordering and a codon ordering of the amino acids. Since the former ordering is based upon pyridine-amino acid interactions, we feel the conclusion is essentially unavoidable that the codon assignments manifest an underlying codon-amino acid pairing. The only question in this regard seems to be whether or not all of the codon assignments were fixed *absolutely* by codon-amino acid pairing (the alternative being that some are fixed only within limits by this interaction).

The approximate number of pyridine molecules bound to an amino acid: If there

is to be a basis for the genetic code in codon-amino acid pairing, it should be possible to demonstrate, among other things, that an amino acid can indeed react with a sufficient number of bases to make its interaction with a "codon" feasible in some respects at least.[13] A rough estimate of the number of pyridine molecules an amino acid will bind can be made as follows: We know that 2,6 DMP reacts little or not at all in a polar fashion with amino acids. For the moment then, assume DMP to be *totally* unreactive with amino acids. Thus, in a mixture of 2, 6 DMP, P, and water, the DMP would serve merely as an inert diluent of the other constituents. By holding the mole fraction of water constant for such solvents, changes in amino acid R_M's as a function of mole fraction of P should straightforwardly reflect the number of pyridine molecules the amino acid will bind. The slope of a plot of log ΔR_M vs. log mole fraction P—analogous to the R_M-water plots—gives that number.[14] Figure 2 shows the results of this sort of experiment—an amino acid appears to bind 3–4 pyridine molecules. This number is about the same for all amino acids tested. (It is not possible to test certain amino acids at low enough water mole fractions, as their R_F's become themselves too low.) If our original assumption about DMP's being totally inert is incorrect, then as is readily seen, the true number of pyridines bound to an amino acid will be *greater* than this apparent number. Now, in the present context, it is not particularly important to determine the exact number of pyridines an amino acid will bind; it is sufficient merely to show this number to be large enough that the possible binding of a trinucleotide is not ruled out.

Discussion and Summary.—The data presented have carried us part of the way to rationalizing a genetic code based upon codon-amino acid-pairing interactions. The *existence* of interactions between amino acids and organic bases closely related to pyrimidine is firmly established. These interactions have both *polar* and *nonpolar* aspects, which are to a small extent now characterized. Amino acids have been shown to interact with 3–4 (or possibly a few more) pyridine molecules, by analogy making an amino acid interaction with a trinucleotide not infeasible. And, a characterization of amino acids in terms of their relative "polar requirements" shows a remarkable resemblance to the ordering of amino acids by the codons to which they are assigned. All in all, the conclusion that the genetic code derives basically from some sort of codon-amino acid-pairing interactions, is essentially unavoidable.

While we think that the facts presented here sketch out the answer to the fundamental question of the genetic code—what mechanisms, etc., give rise to codon assignments—they seem to have raised more questions than they have answered. For example, how could such weak interactions, undoubtedly showing nothing resembling an all-or-none specificity, produce unambiguously assigned codons? Can such interactions catalyze peptide bond formation? What is the geometry of the codon-amino acid complex? How do such interactions fit into the biological context—do they still play an important role in "modern" cells or were they confined to the recesses of evolution? Did life arise in a nonaqueous or semiaqueous environment? We shall have to postpone discussion for most of these points, but we should like to speculate briefly here on some features of the postulated codon-amino acid complex.

In spite of a paucity of data, certain salient characteristics of codon-amino acid-pairing interactions appear to manifest themselves and suggest the outline of a "Codon-Amino Acid-Pairing Model." The relevant facts and correlations are these:

Changing one pyrimidine to another, or one purine to another, in the III position of a codon almost never changes that codon's amino acid assignment. A change from pyrimidine to purine in the III position often does change a codon's assignment, *but* the two amino acids so grouped are always *closely related* to one another (as defined by polar requirement). It is also clear that changing the base in the I codon position—though having more of an effect than a change in the III position—still changes codon assignments only within a group of closely related amino acids, as Table 1 indicates. (Right now this appears to be true perhaps for just the U_{II} and C_{II} codons.) It is only in the II codon position that a base change appears to exert a drastic effect on codon assignment. Thus (realizing that the "codon" in which we are interested may be some transformation of the codon seen in mRNA), we postulate the following: (1) The general geometry of the codon-amino acid complex is basically similar for all codon-amino acid pairs.[15] (2) There exists a hierarchy of the positions in a codon, defined both in terms of kind of interaction (polar vs. nonpolar) and strength of interaction: the II position base is taken to interact strongly with the amino acid R group and is the major base with respect to determining the amino acid assignment of a codon. The I position base is seen as a perturbation on the interaction of the II position base, thus permitting a distinction among the similar amino acids crudely grouped by their interaction with the II position base. For the U_{II} and C_{II} codons at least the I position base interacts much less strongly with the amino acid than does the II position base. The III position base interacts the most weakly of all with the amino acid, and so plays a minor role in fixing a codon's assignment. (3) Codons and amino acids pair according to some sort of "optimization of their energy of interaction," so that all amino acids (within certain subsets of the whole set at least) must have approximately the same energy of interaction with their codons. Therefore, val, for example, which cannot interact as strongly in a nonpolar fashion with the II position base as does phe or leu, compensates by interacting more strongly with its respective I position base (most likely due to the properties of the I position base) than do these other amino acids. Certainly this is not the only model consistent with the available facts, but, nevertheless, it should serve its main intended function, that of a working hypothesis.

The present model, and others for that matter, explains in a very plausible manner why a certain 20 amino acids are used in protein synthesis and not others. The main thing to recognize is that in an important sense, the codon "chooses" its amino acid, not the reverse. Thus, those amino acids assigned to U_{II} codons are very similar to one another precisely because they all correspond to (match) some features of their respective II position base. Amino acids not assigned to codons should therefore be those not properly matching the critical properties of codons. So far, we can point to at least two examples conforming to this expectation, α-amino-n-butyric acid and allo-thr. Both amino acids show polar requirements that are higher than those for U_{II} codons (whose range is 5.2 ± 0.4) and lower than those for C_{II} codons (range 7.0 ± 0.5).[11] (However, allo-ilu may not fit such an explanation, so other features of the geometry of codon-amino acid complexes might play a role in this case.[11])

Finally, if our model is correct, the characteristics of an amino acid should define exactly characteristics of the codon with which it pairs. These latter characteris-

tics in turn could be used to define properties of the codon's component bases. It is of interest then to compare some of the properties that can in this way be predicted for the bases with those which are known. It addition to adding confirmation to some of the above speculations, such a comparison could be of help in defining the relationship between what we mean here by a "codon" and the customary codon appearing in mRNA. Considering first the II position in the codon, on the basis of polar requirement data and our model, U_{II} should be less polar than any others of the bases. The I position in the codon tells us even more about base ranking. According to Table 1 the amino acids with A_{II} codons are ranked (by increasing polar requirement): tyr (U_I) < his ~ gln (C_I) < asn ~ lys (A_I) < asp ~ glu (G_I). Data presented elsewhere also permit the exact ranking of U_{II} amino acids by polar requirement: phe (U_I) < leu (C_I) < ilu(A_I) < met(A_I) < val(G_I).[11] C_{II} amino acids rank, pro (C_I) ~ [thr (A_I)] < ala(G_I) < [ser(U_I)].[16] There is a reasonably clear indication for a I position base-ranking U < C < A < G.

The correspondence between these predicted orderings of bases and some of their actual properties is gratifying. U is clearly the worst electron donor of all the major bases. This can be seen in the low slopes of its R_M-water plots, and by its being the only base not to bind Cu^{+2}, a good Lewis acid.[11, 17] Further, Hückel calculations rank the bases as follows: as π electron donors U < C < A < G; as ring nitrogen electron donors ("average") U ≪ G < A < C; and as O electron donors U < C < G.[18] Although all this does not permit us to claim perfect agreement between prediction and known characteristics, the resemblance between the two is outstanding. Thus it appears that the "codon" which is predicted to pair with the amino acid may turn out to have the same over-all composition as the corresponding codon in mRNA.

Note added in proof: Recent data have shown the differences in polar requirement between cys and try—which constituted an exception to the III position "relatedness" rule—to result from an artifact of cys chromatography in P, MP, and DMP. By properly protecting cys, or by determining polar requirements in pyridine solvent systems which do not create this artifact, it can be shown that cys in actuality has a polar requirement very close to that of try, as we would have expected.

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[1] By "steric interactions" we simply mean interactions where an oligonucleotide shows a preference for interacting with particular amino acids; and which amino acids are preferred is a function of the composition of the oligonucleotide.

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[12] We use the now-customary three-letter abbreviations for the amino acids: phe for phenylalanine, gln for glutamine, ilu for isoleucine, etc. For the bases we use U for uracil, A for adenine, etc.

[13] We shall use "codon" here to mean some oligonucleotide which is simply related to, but not necessarily the same as, the trinucleotide in mRNA usually referred to as a "codon."

[14] $\Delta R_M = 1/\Delta R_F - 1$, where ΔR_F is the difference between R_F in any given P-DMP-water mixture and the R_F in DMP-water alone (same mole fraction of water in both cases).

[15] Although base-base interactions within the codon could in certain cases distort the geometry considerably.

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*HETEROGENEOUS RNA'S OCCURRING DURING THE REPLICATION OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS**

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Brown and Cartwright[1] reported recently that three peaks of virus-specific RNA were found in hamster kidney cells after infection with foot and mouth disease (FMD) virus. When the isolated RNA was centrifuged on sucrose gradients, the bulk of the infectivity was associated with the fastest-sedimenting RNA. However, the two slower-sedimenting RNA's were also infectious, and some of the infectivity associated with these RNA's remained after incubation with RNase, unlike that of the fast-sedimenting peak. The question whether both of the two slower-sedimenting peaks consisted of molecules of double-stranded RNA has not yet been settled. Thus, in addition to the two sedimentation kinds of RNA (i.e., presumably the major strand and the replicative form) found with several other viruses,[2-11] FMD virus added a third. We wish to report here studies performed with Western equine encephalomyelitis (WEE) virus, an RNA virus. Our findings also show that there are three species of infectious RNA formed during the replication of WEE virus.

Materials and Methods.—Cells and virus; Primary cultures of chick embryo (CE) cells were prepared according to the method of Dulbecco and Vogt,[12] and grown in Eagle's medium with 3% calf serum. The source and preparation of WEE virus, its storage, and the assay of infective titers have been described.[13] The WEE virus used was purified by two successive single-plaque