pigs, hamsters, gerbils and mice, did not cause in these animals any pathologic manifestation and was not isolated from their blood or from their organs. When inoculated into frogs, the leptospira only caused a slight and inconstant antibody response. It could not be recovered from the organs of these amphibians.

Serological tests were executed, by the agglutination test, comparing the strain isolated to leptospiras belonging to 13 pathogenic serotypes and to a strain of the 'biflexa' complex. It was not possible to ascertain any consistent antigenic affinity between the strain of the frog and the leptospira strains compared to it.

It is interesting to point out that the serum of the frogs constituting the pool from which the leptospira has been isolated did not contain any agglutinins for this strain.

We have received the strain in question, marked I.C.F. from Dr. DIESCH, whom we warmly thank for his kindness. The strain has been maintained here in Korthof-Babudieri's medium.

Firstly, we have submitted the strain to the biochemical tests which permit us to differentiate pathogenic leptospiras from saprophytic ones. More precisely, the strain has been inseminated in medium containing 8-azoguanine (420µ/ml) copper sulphate (1:100,000) and peptone plus bicarbonate, according to MAZZONELLI[4]. The leptospira has not developed in any of these media, this behaving like a typical pathogenic leptospira.

The leptospira has been inoculated i.p. into young guinea-pigs. They did not show any sign of disease. It was recovered from the blood 1 h after inoculation; not later. It could not be recovered from the liver or kidney of the animals sacrificed after 7 days.

Later on, after preparing with it an immune serum at a high titer (1:1,000,000), the leptospira under study was compared, by cross-agglutination test, to the reference strains of all the serotypes of pathogenic leptospiras known so far[5], as well as to a leptospira strain recently isolated in the Philippines Islands, from the kidneys of a toad (strain 3-C) and still under study. The strains employed in this test were, altogether, 141. None of these strains was found to have a significant antigenic affinity with the strain I.C.F. It only showed a very slight affinity with the *javanica* serotype. Consequently, we can affirm that the strain I.C.F. belongs to the group of pathogenic leptospiras and that it represents in this group a new serogroup and a new serotype, for which we suggest the name '*ranarum*'.

It is interesting to realize that amphibians may be carriers of pathogenic leptospiras. However, in the case we are examining, the scarce virulence shown by the strain I.C.F. for common laboratory animals and even for frogs, and the fact that it belongs to a new serotype, which so far has never been acknowledged to be responsible for cases of leptospirosis in human beings or in domestic animals, makes us presume that the epidemiological importance of this leptospira is very limited.

Zusammenfassung. Nachweis, dass Nieren von *Rana pipiens* Träger pathogener Leptospiren sein können und dass in deren System mit dem neuen Leptospirenstamm eine bisher unbekannte Serogruppe aufgefunden wurde, für welche der Name '*ranarum*' vorgeschlagen wird.

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THEORIA

On the Molecular Mechanism of Action of the Tetracyclines

The tetracyclines are known to exert their antibiotic activity by binding to the 30 S ribosome and blocking the binding of aminoacyl t-RNA[1]. Since the ribosomes are constructed of RNA and protein it may be possible to suggest the nature of the binding site of tetracyclines by a comparison of molecular models of the drug with possible variations of RNA, protein or RNA-protein structures available to bind the small, complex and fixed molecule of a typical tetracycline.

Material and methods. As described in a previous communication[2] Corey-Pauling-Kaltun models of a variety of tetracyclines were prepared as well as segments of various RNA and protein structures and their relationships examined.

Results. The –OH, =O, –OH grouping common to all tetracyclines is complimentary to the NH, NH, O grouping of an Arg-Glu ionically bonded pair or to the N:, NH, O grouping of G-C (minor side) and A-U (major side) base pairs. Therefore, as working hypotheses we set up 1. two protein segments (β-pleated sheet conformation), joined by 2 Arg-Glu ionic (double resonating) cross links, and 2. a segment of RNA with various base pairs. We could observe no particular relationship between the tetracycline molecule and structure 1, but a close relationship became apparent to a fully extended segment of RNA (double strand) of sequences GC:GC or GC:AU as shown in Figures 1 and 2. The tetracycline molecule sits on the minor groove side and forms a roof over the gap between the separated base pairs. It binds as follows (hydrogen bonds except where stated: the numbers refer to the bonds marked in Figure 1): **1**, 10 OH group to cytosine 0; **2**, 11 = 0 from guanine NH; **3**, 10 OH to guanine 3*N*; **4**, the OH between C_{12} and C_1 to ribose ring 0; **8**, the basic NH^+ to phosphate O^- (ionic); **7**, the 3OH to the G (or A) 3*N*: of the second pair; **5**, the 2 C=O from the ribose 2OH of the second pair purine base (weak interaction); **9**, the 6OH to C=O (or U=O) of the second pair and (**10**, **11**, **12**) the C7, 8, 9 hydrogen (and 7 Cl of chlortetracycline and demeclocyline) make lipophilic contacts with ribose 1 and 4 CHs of the second pair and the ribose 4CH of the first pair, respectively. The NH_2 group **6**, (or NH.CH_2.N (C_4H_8) group of rolitetracycline) intercalates between the purine π clouds. There is nothing to indicate whether the purine of pair 2 is guanine or adenine since the 5 position

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of the tetracycline is some 1/2 Å out of contact with the 2 position of the purine. The OH in this position in oxytetracycline, methacycline and deoxycycline is not within H-bonding distance from the guanine 2 HN-group and the CH in this position in tetracycline, rolitetracyline, chlortetracycline and demeclocycline is likewise not in lipophilic contact with the 2CH group of adenine.

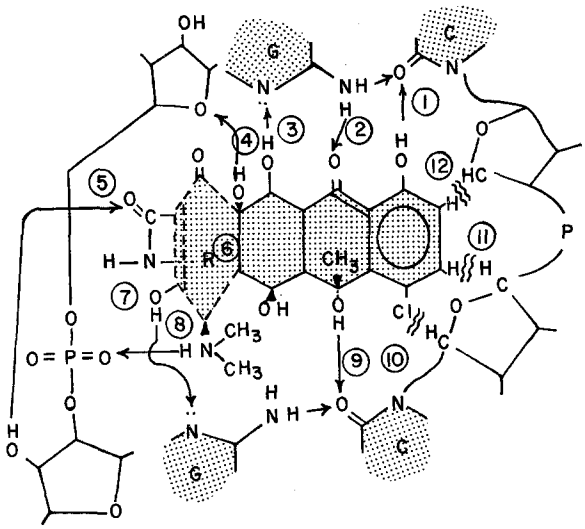

Fig. 1. A composite tetracycline (including all the active groups of the tetracyclines listed in GOODMAN and GILLMAN[4]. The suggested mode of binding is indicated as numbered in the text. The molecule is turned over relative to its normal presentation in line formulae.

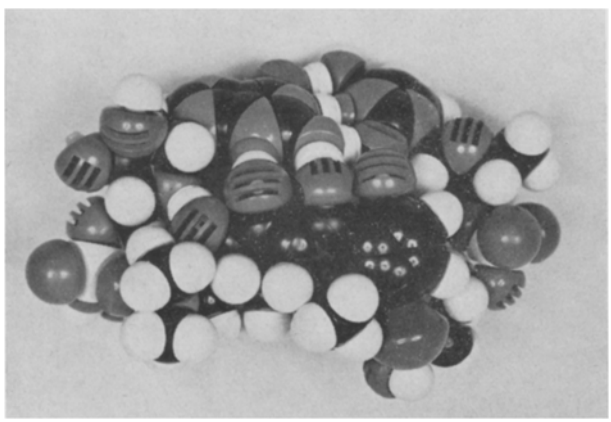

Fig. 2. A CPK model of the proposed mechanism of binding of tetracyclines to the ribosome.

The general 'fit' is extremely precise with correct bond angles maintained in all cases. Every OH group is used as an H-bond donor. The only groups not actively involved in hydrogen bond or ionic interactions are the $C_1 = 0$ and the amide group on 2 (these groups make a van der Waals' contact with the purine π clouds). Thus the tetracycline binds by 6 hydrogen bonds, 1 ionic bond and various lipophilic bonds and weak interactions.

An alternative site is provided by a strand of RNA in the fully extended conformation (2Gs) bound to protein (-Glu-x-Glu-) by 2 G-Glu ion-dipole bonds. In this case the 10 OH and 6 OH bind to Glu Os and C7 and 9 lipophilic bonds are to Glu methylenes and the C8 lipophilic bond to the α CH of x.

A complex protein structure could be derived based on 2 parallel β-pleated chains cross linked by one Arg-Glu link plus one or more additional chains to provide binding sites for the 12–1 OH, the basic NH^+ and the 6 OH groups which cannot be accommodated on the first structure.

Discussion. This precise fit described is not attainable on any other structure involving RNA. Amanatin has a similar precise stereochemical relationship to a segment of RNA (or complexed individual nucleotides) bound to protein[3]. The RNA sequence required is 3 adenines and the protein sequence is -Gln-x-Gln-x-Gln-. In this case double-stranded RNA will not do. Ribosomes could also have a segment of RNA bound to protein in a comparable manner (G-Glu in place of A-Gln). Alternatively the 'receptor' for tetracyclines could be constructed entirely from RNA (double stranded GC:GC or GC:AU). This structure could be located in or adjacent to the binding site for aminoacyl t-RNA so that occupancy by the tetracycline blocks the binding of the latter. It would be of interest to explore the relationship between the structures we have described and t-RNA.

Résumé. L'usage de modèles moléculaires CPK permet de comparer la structure des molécules de la tétracycline à celle des molécules du RNA. Il est suggéré que le site récepteur de la tétracycline sur le ribosome comprend soit du RNA à double tresse, dont les radicaux formés par GC/GC ou GC/AU, soit un complexe RNA/protéine stéréochimiquement analogue au précedent ou à la portion CC (ou CU) du RNA. Il peut enfin être remplacé par une portion isomorphique de protéine ayant une séquence aminoacidique telle que Glu-x-Glu (ou Glu-x-Gln).

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A Model For Investigating Erythropoiesis[1]

Basic models showing the feedback circuit responsible for the regulation of red cell production, have been constructed from the results of investigations concerning erythropoiesis. The models are continually being modified[2–4], since certain factors, once thought to be the source of erythropoietin have no erythropoietic activity[5].

Although the role of erythropoietin in the regulation of red cell development is well established, the processes of production and release remain unclear. The results of early investigations suggested that erythropoietin is produced by the kidney[6,7], and that its site of action is the bone marrow stem cell[8,9]. It is now thought that erythro-