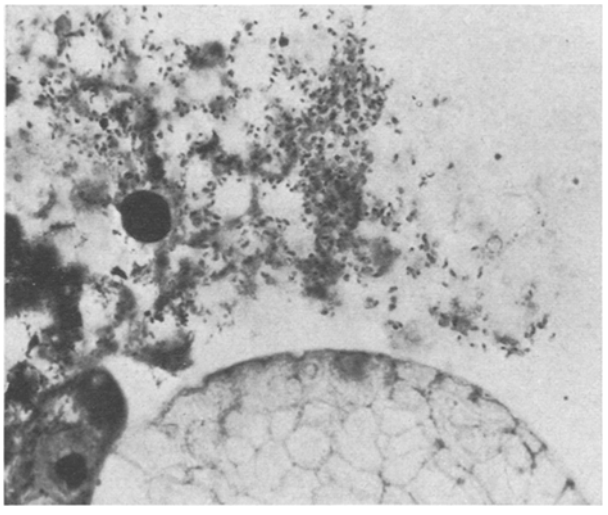

Fig. 2. Agglomeration of sporozoites of Nuttallia meri at the periphery of a salivary gland of O. erraticus. Domed, vacuolated tissue is zymogen-secreting cells. Sporozoites are mainly found adjacent to these. Flattened whole gland preparation, Giemsa-stained, ×750.

solid round specimens, and others containing a vacuole, like young malarial trophozoites, were also encountered in some preparations (figure 1). Masses of sporozoites were seen mainly on the periphery of salivary glands (figure 2) adjacent to the clear zymogen-secreting cells. They were also found within the gland tissue proper and within the saliva collecting tubules. Saliva collected from infected ticks contained sporozoites. Infected ticks transmitted N. meri to clean, laboratory-bred Psammomys and hamsters by bite, and triturated pooled infected glands, injected i.p. into splenectomized clean recipients, also effected transmission. There is a clear difference in the rates of infection, as manifest by the presence of sporozoites in the salivary glands, between ticks that had fed on infected Psammomys and those fed on infected hamsters. Of 309 clean ticks fed on Psammomys, 34% were found positive, as against 18% of 72 clean ticks fed on hamsters.

Interest in O. erraticus, small race, as a vector of Nuttallia meri stems from its being the first Argasid (=soft) tick to have been proved to transmit a piroplasm by bite[3,4]. The vectors of all known piroplasms of domestic animals (both Theilerias and Babesias) belong to the Ixodidae or hard ticks[5–10]. Although several piroplasms of small mammals have been described, their use as laboratory models for the diseases caused by piroplasms of economic importance is limited by the fact that the natural vectors of most are as yet unknown. Only in 2 instances: N. danii Tsur, Hadani and Pipano, 1960, (Babesia merionis (Rousselot, 1953) Levine, 1971) and N. microti Coles, 1914, are these known[11,12]. They are 3 species of Hyalomma and 2 species of Rhipicephalus for N. danii and Ixodes trianguliceps for N. microti, all Ixodid ticks.

The demonstration of sporozoites as the infective form of N. meri in O. erraticus clearly amplifies the contention[4] that the unidentified natural vectors of some of the small mammal piroplasms ought to be sought for among the Argasidae.

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Transcription spectra in E. coli growing rapidly or slowly

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Summary. The percentages of DNA transcribed by Escherichia coli WWU growing with doubling times of 26 or 56 min were examinated by RNA: DNA hybridization. The data indicate that more DNA was transcribed by the faster-growing cells (9.6 ± 0.3%) than by the slower-growing cells (2.4 ± 0.3%).

E. coli grow more rapidly in enriched medium than in unsupplemented minimal medium. The faster growth rate has been related to increases in DNA per cell[2] or to increases in ribosomes per cell[3] by mechanisms supposing that a smaller fraction of DNA is transcribed in the richer medium. This narrower transcription spectrum might be expected since some genes would be repressed by exogenous metabolites. However, in this communication, we report the results of RNA-DNA hybridization experiments which indicate a broader transcription spectrum in cells growing more rapidly.

Methods. DNA labelled with ^{3}H-thymidine was extracted from E. coli WWU[4] after the culture was starved of a required amino acid (arginine) for 60 min to allow completion of cycles of DNA replication in progress. The cells were lysed in an EDTA-saline solution by a freeze-thaw, lysozyme procedure and the DNA extracted by the method

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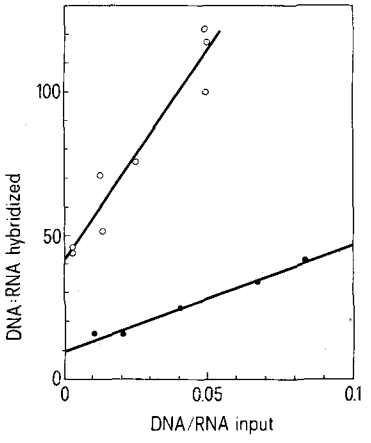
of Marmur[5]. The DNA was denaturated by alkaline treatment (0.9 M NaOH for 60 min at room temperature), neutralized with HCl and stored frozen. The single-strand state of these preparations were verified during the course of the studies by hydrolysis with the single-strand specific endonuclease from Neurospora crassa[6]. Degradation of the preparation was identical to that of DNA freshly denatured by a temperature shift. RNA uniformly labelled with ^{32}P-orthophosphate was extracted from cells growing either in a low-phosphate (1 mM) tris-buffer (pH 7.5) supplemented with glucose and the required metabolites (thymidine, uridine, arginine, methionine, proline and tryptophan; doubling time = 56 min) or in the same medium containing 8 g Difco Nutrient Broth per liter in place of the low-phosphate (doubling time = 26 min). An exponential phase culture was poured into 2 volumes of ice-cold ethanol, concentrated by centrifugation and bacteria were suspended in buffer to about 10^{10} cells/ml. After incubation with lysozyme (5 mg/ml for 1 min at 37 °C), sodium dodecyl sulfate (1.5% w/v) and diethylpyrocarbonate (0.03 ml/ml) were added and the lysate was incubated for 15 min at 37 °C to inactivate nucleases[7]. The RNA was purified by the method of Summers[8]. At least 97% of the acid-insoluble radioactivity was sensitive to RNAase or alkaline hydrolysis. Individual RNA preparations had specific activities in the range of 19–102 million cpm/mg, depending on the amount of ^{32}P-orthophosphate added to the growth medium.

RNA:DNA hybrids were allowed to form in solution in 6 × SSC at 66 °C[9]. Assays for loss of acid-insoluble radioactivity during incubations as long as 60 h showed no degradation of RNA nor DNA. Samples were collected on nitrocellulose filters (Schleicher and Schuell, type B-6), washed extensively with 2 × SSC, challenged with RNAase to reduce contributions from non-specific absorption of RNA (20 μg/ml in 2 × SSC for 60 min at room temperature) and again washed. Retention of the DNA varied around 90% of input, with the lowest value greater than 75%. Retention of RNA by control filters (resulting from incubation of RNA without DNA) varied around 0.028% of the input with extreme values of 0.003% and 0.063%. A melting profile of hybrids in 0.5 × SSC showed abrupt release of RNA from DNA at 72 °C, with at least 70% of the transition occurring between 70 and 74 °C.



Determination of the fraction of DNA transcribed. DNA (2–10 μg/ml) was hybridized with several concentrations of RNA as indicated in text. DNA/RNA input values were plotted against corresponding DNA:RNA hybrid values obtained with RNA from bacteria growing rapidly (●—●) or slowly (○—○). Straight lines were fitted to each set of data by the method of least squares with correlation coefficients of 0.994 and 0.965 respectively. The reciprocals of the average intercept values obtained from 3 such experiments corresponded to 9.6% (●—●) or 2.4% (○—○) of the DNA transcribed.

Results and discussion. Typically, hybridization reactions reached plateaus after incubations of about 30 h. However, plateau RNA:DNA hybrid values varied with the RNA/DNA input ratio. Where both RNA and DNA are free to migrate in solution, a given single-strand sequence of complementary DNA may associate either with RNA or with DNA. Thus the hybridization ratio, RNA:DNA, would depend on the fraction of the DNA which is complementary to RNA (F) and the probability of an association with RNA rather than with DNA. The latter may be approximated by the ratio: RNA/(RNA + P × DNA), where P is an adjustment factor to include both the fact that only one half of the DNA is complementary to the other, and that there are differences between RNA:DNA and DNA:DNA nucleation processes. The reciprocals of these terms yield an equation similar to that introduced by Bishop et al.[10] for hybridization with DNA immobilized on a filter:

$$\text{DNA:RNA} = \frac{1}{F} + \frac{P}{F}\,(\text{DNA/RNA})$$

To estimate the percent of DNA homologous to RNA (i.e., when there is saturation of all complementary DNA sites with RNA), double reciprocal plots of plateaus obtained for RNA:DNA hybrids against RNA/DNA input ratios were examined (figure). Straight lines fitted the data reasonably well and saturation (intercept) values were determined. The results of 3 independent experiments uniformly suggested that a larger fraction of DNA was transcribed by by E. coli growing more rapidly. RNA from rapidly-growing cells gave an average saturation value of 9.6%, whereas RNA from slowly growing cells gave an average value of 2.4%. This difference is significant, although the values are probably underestimates of the actual transcription spectra. Bishop et al.[10] have shown that the extent of hybridization depends in large part on the frequency distribution of an RNA population; some relatively infrequent RNA species might never be detected.

Our data suggest that in faster-growing cells additional functions are expressed. Therefore, one must appreciate a balance in bacteria between the repression of certain pathways to eliminate unnecessary functions and the expression of other functions to exploit a more nutrient situation. The net effect is apparently more extensive use of genetic information by bacteria growing more rapidly which may be appropriate for the additional metabolites, or which may be necessary to increase metabolic capacity at rate-limiting steps.

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