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Ecological Ectocrines in Experimental Epidemiology

A new class, the "pacifarins," is delineated in the nutritional ecology of mouse salmonellosis.

Howard A. Schneider

The general subject of the nutrition of hosts as a factor in resistance to infectious disease has hardly languished for want of discussion (see *1, 2*). But I think it may be fairly asked whether the accomplishments in this area are comparable to those of modern scientific nutrition in some other areas of public health. The reason for my skepticism is this: If nutrition is to be viewed as being effective, either theoretically or practically, as a means of coping with infectious disease, then it inevitably must be judged in the light of other theoretically fruitful and demonstrably successful measures, such as sanitation, vaccination, and antibiosis. In this comparison, it must be admitted, nutrition suffers.

But if, in this context, nutrition is relatively downgraded both by the theoretician and by the decision maker in the field of public health, why is there a periodic resurgence of interest in, and discussion of, nutrition and infection? This perennial florescence of interest and discussion follows, I think, from several considerations, some of them not strictly scientific. To ignore them, however, would be unscientific. Apologists for nutrition as a factor in resisting disease draw attention, for example, to the historical connection between famine and pestilence. This association, however, may be a matter more of concomitance than of causality. Results of modern scientific studies of nutrition in animals suggest that in nutritional science we may have an instrumentality not yet used widely enough or discerningly enough for our ends. These new studies raise newer hopes. Again, modern nutritional science has led to the control of certain well-defined diseases, such as scurvy, beriberi, pellagra, rickets, and some of the anemias. The continuing hope of similarly and nutritionally controlling other diseases, such as coronary thrombosis, hypertension, allergic states, and mental abnormalities, spurs investigators to wrestle once again with an old problem.

With this cursory synopsis of the contemporary mood and the past history of the problem, an explanation for any attempt to inject fresh meaning into the subject would seem in order. My explanation is this. Through circumstance I have been able to sustain investigation of the problem of nutrition and infectious disease for a relatively long time (*3*), and certain features and results of the investigation to date move me to take a new and hopeful view. After long years of doubting, I now assert (i) that a specifiable relationship does exist between host nutrition and response to infectious disease; (ii) that I have reached this conclusion through animal experiments based on a new and unique experimental design; (iii) that this design follows from an analysis of the problem in ecological concepts that go beyond classical concepts in the fields of nutrition and microbiology; (iv) that, in the case of mouse salmonellosis (an infectious disease model), an organic compound representing a new class of compounds has been discovered in the nutritional environment, which, when ingested in very small amounts, greatly increases the host's chance of survival; and (v) that this new compound has features similar to those of ecological ectocrines.

Historical Origins of the New Approach

Investigations into infectious disease classically have their origins in the field. The investigation discussed here originated, not in field studies, but in what was once known as "experimental epidemiology"—that is, the study in the laboratory of a "natural" infectious disease in populations of experimental animals. The notion of experimental epidemiology clearly can be traced to a proposal by Topley in his Goulstonian lecture of 1919 (*4*). What Topley proposed was not the use of the lab-

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Table 1. The a priori parameters in the infectious disease model (mouse salmonellosis).

Host factors	Pathogen factors
1. Temperature	1. Dose
2. Humidity	2. Route of administration
3. Lighting	3. Culture of pathogen
4. Caging	(i) Medium
5. Age	(ii) Age
6. Sex	
7. Previous exposure to pathogen	
8. Genetic constitution	
9. Nutrition	

oratory in searching for and investigating microbial etiological agents of infectious disease (an obviously well-established practice by then) but its use for a deeper biological analysis. His proposal was based on the supposition that there exist certain general biological features which lie behind all epidemic infectious disease and that these features might best be brought to light in the controlled laboratory study of a few "natural" infectious diseases in populations of small, easily housed animal species, such as the mouse. Topley's suggestion was a response to the profound challenge of the great influenza pandemic of 1918–1919. At that time, etiological investigations of influenza had become bogged in claims and counterclaims; Topley was, it seems to me, striking out boldly to find a way out of the mire by rejecting influenza *qua* influenza and readdressing himself to epidemics *qua* epidemics.

Table 2. The experimental diets. (With both diets the animals were given all the distilled water they wanted.)

Component	Amount	
	Milligrams	Grams
Natural diet (diet 100)		
Ground whole wheat		66
Dried whole milk		33
Sodium chloride		1
Semisynthetic diet (diet 191)		
Casein (Labco, vitamin-free)		18.0
Glucose (cerelose)		72.55
Salts W-2		4.0
1-Cystine		0.2
Water-soluble vitamins:		(0.25)
Thiamine hydrochloride	2.5	
Riboflavine	5.0	
Pyridoxine hydrochloride	2.5	
Calcium pantothenate	10.0	
Nicotinic acid	25.0	
Choline chloride	100.0	
Para-aminobenzoic acid	5.0	
Inositol	100.0	
Fat-soluble vitamins, in cottonseed oil (Wesson):		(5.0)
β-carotene	0.72	
Viosterol*		
2-Methyl-1, 4-naphthohydroquinone diacetate	0.33	
α-Tocopherol acetate	11.7	

* 170 I.U.

Topley founded an English school of experimental epidemiology at the London School of Tropical Medicine and Hygiene, and an American school of similar studies was soon established, in the early 1920's, at the Rockefeller Institute for Medical Research in New York, under the leadership of Leslie T. Webster. In a sense both of these enterprises, at least in the form given them by their founders, ended in 1943 with the death that year of the two innovators. The English school published a monograph (*5*) which summarized its findings. Webster was preparing a similar publication at the time of his death (*6*). Twenty years later, it must be acknowledged, the impact of Topley's bold suggestion has all but disappeared. Topley's and Webster's experiments were not performed in obscurity, and there are undoubtedly reasons for the present neglect. I suggest that it stems from the simple fact that the high hope of Topley and his coworkers that some new biological principle responsible for the genesis of epidemics, *any* epidemic, would be brought to light was not realized. Much was learned, and valuable techniques were developed, but in the end the fascinating events of Topley's laboratory-mouse "epidemics" were analyzed in terms and concepts already well recognized and in the forefront of epidemiological thought.

An appeal to "natural immunization" set the interpretation of epidemic events by the English school apart from that of the American school. Webster's experiments with mice and "mouse typhoid" (salmonellosis) had driven him slowly away from a preoccupation with the causative microorganism and the immunological responses it evoked. Instead he had come to focus on features of the host's natural resistance, the differences in response to infection observed in host populations when these encounter the pathogen for the first time. With the techniques of mammalian genetics—that is, through inbreeding and selection by testing litters without exposing the parents—he showed that a mouse population could yield inbred lines widely divergent in their response to infection, by mouth, with *Salmonella* (*7*). From such genetic stocks he assembled herds of differing composition, comprised of mice from the resistant and susceptible lines in various proportions. When such herds were exposed to infected immigrant mice, the results were those predicted; the resistant mice survived and the susceptible mice died (*8*). This demonstration of the role of genetic factors in predisposing mice to withstand or succumb to disease following infection under herd conditions was probably Webster's greatest contribution. It was eventually followed by a second important contribution (*9*), demonstration that resistance and susceptibility to one disease (mouse salmonellosis) were independent of resistance and susceptibility to a second (St. Louis viral encephalitis).

The importance of these contributions of the American school of experimental epidemiology can hardly be overestimated, yet Webster's work has not escaped the fate of Topley's studies. It may be, however, that Webster's work was gradually forgotten, not because it failed to generate a new viewpoint in the analysis of the phenomenon of epidemics—as indeed it did—but because it led into a cul-de-sac in terms of public health. If Webster was right it was all very interesting, but there was nothing in his analysis which armed the public health commissioner with tools for doing something about epidemics. And if information is not used, it is likely to be forgotten.

The Nutritional Environment

Neither Topley nor Webster, in their studies of mouse populations and mouse diseases, was unaware of the possibility that the food their mice ate might influence their ability to withstand the infectious diseases to which they were exposed. Both Topley and Webster (*10*), with their co-workers, performed experiments to examine this possibility. The English school concluded that diet had little effect (*11*). Webster, in the 1920's and prior to his demonstrations of the genetic factors, felt that diet was a very real factor, but his specification of possible nutritional factors remained rather vague, and the experimental results themselves, today, appear erratic enough to justify the caution which tempered his enthusiasm. By 1940, however, the field of experimental nutrition had met with a series of successes, and a degree of experimental sophistication had been achieved which promised an increased capacity to resolve old nutri-

tional problems in terms of a newer and more definitive biochemistry.

It was in this climate of renewed interest and hope, in 1940, that Webster resumed his studies on nutrition and infectious disease and invited me to join him as an experimenter in nutritional biochemistry. The history of these investigations may be traced in the series of publications which subsequently appeared (*11–17*) and in several synoptic accounts (*18–21*). In the remainder of this article I deal with those features of this overall endeavor which are, I believe, of interest from the standpoint of the light they shed on certain interdisciplinary theoretical questions.

Model Making

In Table 1 (*21*) are listed the various parameters of host and pathogen which were considered, a priori, to be relevant to a laboratory model of infectious disease. In compiling this list we had to be selective. We ignored magnetic fields and barometric pressure, for example, as factors which might legitimately claim our attention. The experimental conditions (see Table 1, host factors 1–6) were as follows. (i) Temperature, 26.7°C (80°F). (ii) Relative humidity, 50 percent. (iii) Lighting: artificial illumination provided by fluorescent lamps [flux, approximately 25 footcandles (275 lumens per square meter) at the cages]; day length, 12 hours. (iv) Caging: the mice were individually caged on mesh floors in suspended galvanized iron cages. (v) Age: the mice were infected with *Salmonella* when they were between 6 and 7 weeks old. (vi) Sex: both sexes were tested, but most of the work was done with males.

The virulent *Salmonella typhimurium* pathogen populations were cultivated for use in still nutrient broth cultures incubated at 37°C for 18 hours, centrifuged, diluted in sterile pyrogen-free saline, and administered by intraperitoneal injection of 0.25-milliliter volumes containing 1000 viable organisms. The mice were observed for 30 days after injection, and the results had the desired range: some mice died, some mice survived. The proportion that survived was taken as the determinate index of the host status, and it was through changes in this status that the effectiveness of dietary changes was measured.

Diet

When it came to choosing the dietary changes by means of which we sought to alter the natural resistance of the mouse host populations, we again had to be selective. We set aside temporarily, for example, the problem of starvation, and we ignored the innumerable quantitative variations, and the infinite permutations and combinations of the intakes of individual amino acids, carbohydrates, lipids, mineral elements, and vitamins. Instead we chose to study the relative effect on susceptibility to infection of a semisynthetic diet and a simple diet of natural foodstuffs. The composition of these two diets is given in Table 2 (*22*). This choice reveals our fundamental presupposition: that "natural" foods contain some important items that are not yet known and so not supplied by the assembled semisynthetic diet.

Genetic Framework

Among the host parameters thought worthy of inclusion, a priori, in the model was that of genetic constitution (Table 1, factor 8). Its inclusion was, of course, a direct consequence of Webster's work. It was anticipated that Webster's inbred mouse stocks would be ideal material for the investigation because of their genetic uniformity. For completeness, and also because of greater availability, a third kind of mouse stock, a random-bred, nonselected stock of Webster-Swiss mice, was added. In these early experiments (*12*) we were confronted with the finding that the feeding of synthetic and natural diets resulted in differences in the number of survivors only in the random-bred stock (even these differences were modest). The inbred stocks were unaffected by this difference in diet, and the resistant and susceptible stocks respectively survived or died independently of the diet. In a word, the inbred stocks were uniformly resistant or susceptible, and the two different levels of resistance thus represented did not change with change in diet. On the other hand, the genetic resistance of the unselected stock can be thought of as distributed over a continuum with the population clustering into a Gaussian peak; this peak represents a significant fraction of the population, enough to make detectable

Pathogen-Genotype	Host-Genotype: Inbred, selected, resistant	Random-bred, (outbred) non-selected	Inbred, selected, susceptible
Uniformly virulent	N-Died S-Died	N-Died S-Died	N-Died S-Died
Mixed virulent and avirulent	N-Survived S-Survived	N-Survived Dietary effect S - Died	N-Died S-Died
Uniformly avirulent	N-Survived S-Survived	N-Survived S-Survived	N-Survived S-Survived

Fig. 1. The effect of a natural (*N*) and a synthetic (*S*) diet on survivorship following infection in nine different genetic circumstances. [From H. A. Schneider (*19*)]

a toppling of the peak in one direction or the other as a result of change in diet. The responsiveness to diet may well have its basis in the heterosis arising from the multiple heterozygosities perpetuated by the breeding system. As a consequence of this supposition, in 1944 we devised a simple but planned system of randomized breeding (*12*), which has been very useful.

On purely operational grounds, therefore, we abandoned Webster's inbred stocks in these investigations and turned to the outbred, *Salmonella*-free, Webster-Swiss stock. Host factor 8 (Table 1) thus received a new specification.

New and additional genetic choices had to be made when, as a result of the experiences related above, we came to examine the role of genotype in the pathogen population. The operational basis for this new choice in our model is shown in Fig. 1 (*19*). Clonal virulent *Salmonella typhimurium* killed all mice, irrespective of diet or genotype. With identical doses of clonal avirulent *S. typhimurium*, all the mice survived. But when both clonal cultures were used at the same doses as before, but now combined, differences in survivorship appeared. Inbred resistant mice survived, independent of diet, and inbred susceptible mice died, independent of diet. Only in the random-bred, nonselected mice did we obtain survivorship differences attributable to diet. The best model thus had some novel, even heretical, biological features, and the discoveries to which we were led are to be attributed, I believe, to this attention to strategy in model-making rather than to any revolution-

ary departures in tactics, either biochemical, nutritional, or microbiological.

New Tactics

The emergence of a new strategy, outlined above, raised the possibility of developing new tactics for investigating the situation thus uncovered. Two tactical artifices were next developed which (i) increased the power of our model to resolve the relevant features of the nutritional environment and (ii) increased the rate of flow of nutritional information.

The first of these artifices was an exploitation of the polymorphic nature of the useful pathogen population. I found (*13*) that if a short interval (24 to 48 hours) was introduced between injection of the avirulent salmonellae and injection of the virulent salmonellae, then with increase in the superinfection interval, survivorship increased, but differentially; that is, the increased survivorship was observed with both diets but was greater for the wheat diet than for the semisynthetic diet. Analysis of this method of dual infection, with a range of time intervals, is shown, in terms of survivorship, in Fig. 2 (*13*).

It may be seen that, for this particular pair of clonal cultures, interaction of the two cultures in the mouse at time 0 led to the death of all mice, and of course to no survivorship differences attributable to diet. These two *Salmonella* populations, had they been administered only as a mixture at time 0, would have been dismissed as without usefulness in our studies. But Fig. 2 clearly shows the consequences of allowing even 24 hours to elapse between injections of avirulent and virulent salmonellae: an unambiguous survivorship difference attributable to diet was observed. I suggest that such an analysis will always reveal an area of divergence in survivorship for any particular, randomly chosen, avirulent-virulent pair of pathogens, and that a time interval can be found at which the survivorship difference attributable to diet is maximal. Once chosen, such a selected pair of pathogen populations and such a selected interval have provided an infection method which gives very reproducible results. In our experiments the interval was usually 24 to 48 hours.

The second artifice was the outcome

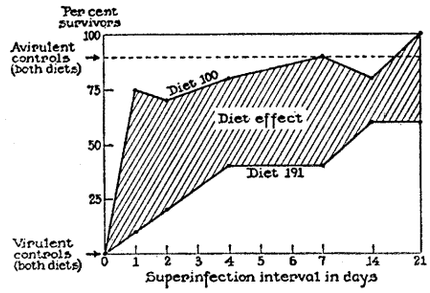

Fig. 2. Difference in survivorship due to diet as a function of the time interval between the challenges with avirulent and virulent bacteria. [H. A. Schneider (*13*)]

of an investigation (*14*) designed to probe more deeply into the nature of the interaction, in the mouse host, between the avirulent and the virulent salmonellae, an interaction which had been shown to be so vital to the detection of the influence of diet on survivorship.

In 1952 I became aware that our particular *Salmonella* populations were lysogenic and that the temperate bacteriophages that the avirulent and virulent salmonellae of our model harbored could be exchanged in vitro. This opened up the possibility that the puzzling interaction of the avirulent and virulent salmonellae had its basis in the phenomena of bacteriophagy, and that these events had significance for the mouse host because of the then newly discovered process of transduction of bacterial genomic character by temperate phages. Although Webster and Topley had both considered bacteriophage in their experimental epidemiology, they had both dismissed

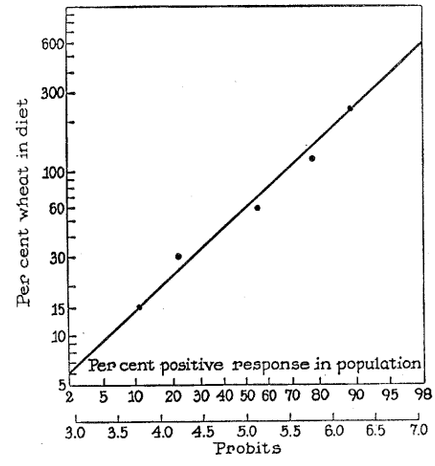

Fig. 3. Survivorship response of the infected Webster-Swiss mouse population to various dietary concentrations of the resistance factor (SRF). [H. A. Schneider (*2*)]

the possibility of its playing any role. But this dismissal was based on the earlier view that all bacteriophages have fully lytic action. The new phenomenology of the temperate bacteriophage was very different.

At this point Norton Zinder, the codiscoverer of transduction in *Salmonella*, joined me in this endeavor. We eventually showed (*23*) that lysogenizing the virulent salmonellae, in vitro, with the temperate bacteriophages resident in the avirulent salmonellae did not change the virulence of the former, or, reciprocally, the avirulence of the latter. In view of the low efficiency of transduction and our inability to screen for the putatively transduced clones, this result was probably to be expected. Finally, great doubt was cast on the hypothetical in vivo role of temperate bacteriophage in the bacterial interaction in our model by our finding that the interaction was unaffected even when we rendered the two bacterial populations involved immune to transducing phenomena by reciprocally lysogenizing them in the test tube with the bacteriophages carried by the potentially interacting bacterial partner.

Zinder and I (*14*) then studied the population kinetics of interaction between the virulent and avirulent salmonellae, in the mouse, by using an indifferent genetic marker, xylose-fermenting ability. A xylose-fermenting mutant was selected out of the original parent virulent *Salmonella* population, the latter being a xylose nonfermenter, as was the avirulent population. The xylose-positive mutant was shown (*14*) to be as virulent as the parental stock. On eosin-methylene blue agar plates containing xylose the xylose-fermenting virulent cells gave rise to opaque black colonies and the xylose-nonfermenting avirulent cells gave rise to white translucent colonies. By this means it became possible, in vitro, to enumerate separately the two components of the mixed populations, and the dimorphism which had been covert (the real basis of the difference in virulence remains unknown) was made overt. It was now possible to analyze the separate kinetics of increase of the two bacterial populations after they had been injected into the mouse. The spleen was chosen as a suitable anatomic site of these events, and it was shown (*14*) that, as predicted, when either bacterial population constituted a single infection its kinetics of multiplication and ultimate fate were un-

influenced by the nutritional environment of the host. However, in the instance of the dual infection, although the avirulent bacteria pursued their usual course, the virulent bacteria now followed one or the other of two different kinetic patterns. In the mice on the semisynthetic diet, the virulent bacteria, in the greater proportion of the mice, tended to multiply vigorously until the host died. But in the mice on the whole-wheat diet, the virulent bacteria tended to conform to the kinetics of multiplication of the avirulent form: after some initial multiplication they subsided into a small, latent population which, with time, became smaller and smaller (*16*). This dichotomy in the kinetic pattern of the virulent salmonellae was, of course, suggestive of the dichotomy in the fate of the mice themselves.

What is worthy of emphasis is the fact that the operation of the diet-supplied resistance factor, with resulting survivorship, was thus seen to be, not a changed average value in a bacterial-population continuum, but a discrete bimodality. One set of this bimodality was associated with the eventual death, and the other set with the eventual survival, of the mouse. The effect of diet was to change the frequency of the two sets. This bimodality was visible as early as the second day after the challenge with the virulent bacterium, and the number of virulent salmonellae present in the spleen of a mouse on that day predicted either survival or death, according to whether the count was low or high, with a very small area of overlap (*14*). The second artifice thus arrived at was the adoption of a bacteriological datum, an appropriately interpreted count of the genetically marked virulent organisms in the spleens of mice sacrificed 2 days after challenge, and the supplanting thereby of the more time-consuming 30-day survivorship experiments.

On the Trail of the Resistance Factor

Modest, but detectable, amounts of salmonellosis-resistance-factor (SRF) activity can be found in wheat, corn, rye, and rice, and in such rather special sources as malted barley sprouts or dried green and black tea. Most of our fractionation studies, however, have been largely concerned with the activity in whole wheat (*15*). The wheat grain can be fractionated mechanically by grinding and sieving. Assay showed that the activity of the wheat was in the outer, 25-percent fraction of the wheat kernel, which resists pulverizing, while the 75 percent of the kernel which comprises the endosperm, white-flour fraction passed through the sieves but lacked this activity. Concentration of SRF by milling and sieving provided an opportunity to construct diets of high SRF activity, and a dose-response curve (Fig. 3) revealed the interesting fact that survivorship, transformed from percentages into population probits, was related linearly to the logarithm of the dietary concentration of the SRF source (*2, 15*). Experiments showed that when animals were shifted from diets containing SRF activity to diets lacking it, and vice versa, the effects of the shift were almost immediate. A mouse which had eaten SRF all of its life, upon withdrawal of SRF responded in 2 days' time as if it had never eaten SRF at any time (*14*). This revealed an extremely dynamic state of affairs.

During the course of the experiments with wheat it became embarrassingly evident that there was considerable variation in the degree of SRF activity in the samples of wheat used. In an attempt to understand this variation, more than 25 varieties of wheat were examined, including emmer and durum wheats and even primitive einkorn. Toward the end of this considerable experimentation with the wheats it became clear that the variation encountered was attributable not to any genetic differences among the wheats but, rather, to some ill-defined variation in their culture. An escape from this frustrating situation was provided when we happened to assay commercial dried egg white. This proved to be a source of SRF activity equal to the best of the wheats. The SRF activity of commercial dried egg white was easily shown not to be due to the protein content, for, as in wheat, SRF activity was extractable with methanol, and the residue was itself inactive.

Work with the wheats was now abandoned in favor of commercial dried egg white. This is a fermented product, and experiments quickly showed that, in contrast, sterile, lyophilized fresh egg whites were inactive. It was next shown that inactive fresh sterile egg whites could be fermented by a dose of the viable complex microflora present in the commercial product. The lyophilized product of a few days' fermentation at room temperature now had SRF activity. Our search became a microbiological hunt for the bacterial species present in the commercial dried egg white which was responsible for the generation of SRF activity consequent to fermentation of the egg white. Most of the bacterial species present were incapable of generating SRF activity on egg white, but ultimately we isolated a species of *Aerobacter* which could do so (*17, 24*). Further difficulties lay ahead, for nutrient broth cultures of the successful *Aerobacter* proved inactive, despite the effectiveness of the *Aerobacter* in generating activity on egg white. Evenutally, this problem, too, was resolved: a simple synthetic medium of sodium lactate, ammonia, and salts, under aeration, allowed biosynthesis of SRF activity by the *Aerobacter*. The activity was found in the medium, and negligible amounts were found in the bacterial cells.

Table 3. Properties of the salmonellosis resistance factor.

1. Destroyed by ashing
2. Soluble in water and methanol; insoluble in a wide variety of organic solvents
3. Dialyzable
4. Heat-stable
   (i) Stable at *p*H 2 to *p*H 10 (100°C for 30 minutes, in water)
   (ii) Destroyed at *p*H > 12.0, room temperature
   (iii) Destroyed by autoclaving in 6*N* HCl
5. Anion at *p*H > 4.0
6. Class reactions:
   (i) Positive Hoepfner and phloroglucinol tests for *o*-dihydroxy phenols
   (ii) Ninhydrin negative, but positive after hydrolysis in 6*N* HCl
7. Forms chelates with metal ions of the first transitional series
8. Four active colored Fe^{+++} chelates recognized: at *p*H 4; red, two violets, blue

Chemical Properties of the Resistance Factor

The chemical properties of SRF as biosynthesized by our *Aerobacter* species are indicated in Table 3. Although investigation of the chemical properties of the SRF found in the wheat

had been in its early stages at the time of the shift to fermented egg white sources, there is some evidence (*25*) of chemical differences between the wheat material and the SRF biosynthesized by the *Aerobacter* in synthetic media. Such differences are, of course, worrisome, but since it is no novelty to encounter biological activity in various chemical forms in nature, we decided to investigate the chemical properties of the SRF obtained from the most reliable source at hand and allow future work to elucidate the differences between SRF's from diverse sources. The *Aerobacter*-derived SRF, then, is the one which has been investigated the most thoroughly. H. N. Wood and R. W. Colburn participated in this phase of our studies. An intriguing chemical property is the chelating ability, which extends to all of the elements of the first transitional series. We have been unable thus far to assign a special role to any of these elements. If any one element claims our attention more than the others it is probably iron. The addition of iron, for example, to the biosynthesizing medium depresses biosynthesis. In our infection model the iron chelate was active, but so was the des-ferri form. Other iron chelates, such as iron-ethylenediaminetetraacetic acid, ferrichrome, ferrioxamine B, and Fe-2,3-dihydroxybenzoyl glycine, were all inactive.

Our best SRF preparations are active enough to excite attention; 200 to 400 parts per billion in the diet raise survivorship in our model of mouse salmonellosis from a base level of 10 percent to 90 percent.

Elemental analysis of our preparations reveals carbon, nitrogen, hydrogen, sulfur, and oxygen, but no phosphorus. We have obtained three active forms which vary in their sulfur content, but since good criteria for chemical purity are still lacking, not much can be inferred from these data as yet. Class-reaction tests performed on our best materials have consistently revealed a catechol grouping and, after acid hydrolysis, some amino acids, especially serine (*26*).

Biological Categorization

Salmonellosis resistance factor is an organic molecule biologically active in minute amounts on ingestion, and is a product of microbial biosynthesis. One immediately wonders, therefore, whether this newly found substance is but a special instance of already well-recognized classes of organic substances of high biological potency. Two such classes spring to mind. Is SRF a vitamin, or an antibiotic? The answer is that it is neither. It is not required by mice or salmonellae for their growth or maintenance, and no bacteriostatic or bacteriocidal effects are demonstrable in any in vitro systems that we have tried. If, by these criteria, SRF is neither vitamin nor antibiotic, what precisely is it? Marine ecology supplies a concept of some relevance. The phenomena of ecological exclusions and successions, for example, are not readily understood on the basis of food supplies in the ocean waters. C. E. Lucas (*27*) has collected evidence to show that these events are in part controlled by minute amounts of organic chemical substances present in traces in the waters as a consequence of the presence of prior inhabitants. These organic substances Lucas has called "ecological ectocrines." I believe it useful to consider SRF to be a new kind of ecological ectocrine. Lucas defines an ecological ectocrine as a chemical substance biosynthesized by one species and exerting an effect on the function of another via the external medium. The notion of ecological ectocrines is thus a very broad one, and it is, I think, interesting that vitamins and antibiotics can be subsumed under it: vitamins are biosynthesized by some species and, when delivered by the environment, can support the life of other species that require them but do not synthesize them; antibiotics are biosynthesized by some microbial species and, when delivered by the environment, can adversely affect other microbial species.

Table 4. Ecological ectocrines. [After Lucas]

Class	Examples
1. Vitamins	A, B_2, C, D, E, K, etc.
2. Antibiotics	Penicillin, streptomycin, terramycin, etc.
3. Pacifarins	Salmonellosis resistance factor (etc. ?)

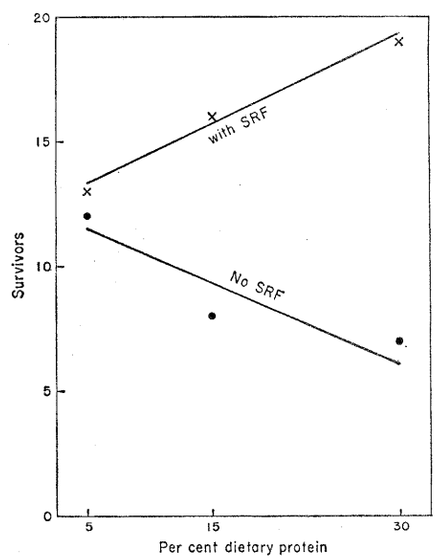

Fig. 4. Divergent effects on survivorship in mouse salmonellosis, demonstrating the interaction between dietary protein concentration and the salmonellosis pacifarin (SRF). Each point represents the response of samples of 20 mice. [Hill *et al.* (*28*)]

But, as I have said, SRF is neither vitamin nor antibiotic. It seems necessary to create for it a new class within the category of ecological ectocrines (Table 4). We have called this new class the "pacifarins," from the Latin "pacificare," to pacify (*20*). Salmonellosis resistance factor is thus the salmonellosis pacifarin and, so far, the only recognized member of this new class. It may be, of course, that other pacifarins will be found, pertaining to other infectious diseases. And what, precisely, does a pacifarin do? Biologically considered, it chemically mediates what the ecologist calls "interspecific nonpredator relationships." There may be subtle intraspecific relationships as well, for we now know that our avirulent salmonellae biosynthesize the pacifarin and that the virulent ones in identical circumstances do not. We can now see how, in the polymorphic *Salmonella* populations of our experiments, there was provided a built-in supply of the pacifarin which rendered our model more sensitive and more responsive to an external input. Apparently, through ingestion of food by the host, concentrations of the pacifarins, which are indigenous in the polymorphic pathogen population and are of great importance in the complex picture of infectious disease, can be increased. Topley said in his Goulstonian lecture that any real understanding of epidemic disease must include an explanation of the way in which the rise of an epidemic brings with it the seeds of its end. The indigenous pacifarins of the polymorphic pathogen population may be that seed, and the pacifarins of the nutritional environment, merely more of that same seed.

Salmonellosis Pacifarin in Nutritional Interaction

Recognition of the salmonellosis pacifarin, and its availability even in crude form, made it possible to design multifactorial experiments involving the pacifarin and such classical nutritional elements as vitamins and proteins. One such experiment, by C. H. Hill, R. W. Colburn, and myself (*28*), provided an insight into a matter which has long been the subject of controversy in the field of nutrition and infection: What is the effect on resistance to infection of an increase in dietary protein? There are three possible answers: increasing the protein intake either (i) increases natural resistance, (ii) decreases natural resistance, or (iii) is without effect. All three answers, supported by experimental evidence, can be found in the literature.

Our experimental results suggest that all three of these replies, seemingly so contradictory, may be correct, depending on whether or not an adequate supply of an appropriate pacifarin is ingested by the host along with the protein. In the mouse salmonellosis model, increasing protein levels in the absence of the pacifarin decreased survivorship. When the pacifarin was supplied, the results were precisely the reverse—survivorship was *increased* (Fig. 4). This is interaction of a very critical kind, for to manipulate protein intakes in an effort to improve natural resistance, but in ignorance of this important role of pacifarins, is to act in a most capricious way. Increased resistance may be achieved, or the results may be just the opposite. Here again there can be little doubt that the pacifarins, interacting so powerfully with the ingested protein, are indeed an appropriate subject for nutritional investigation.

Summary

I have attempted to sketch the ideas forged in a long-term investigation of the possible relationship between host nutrition and resistance to infection. Our endeavor had its origins in the now partially forgotten field of experimental epidemiology, once epitomized in somewhat contrasting schools of thought, English and American. The work of Webster, which showed the importance of host genetic constitution, was an early and decisive influence in the work discussed here, and led to formulation of a new laboratory model of infectious disease, which has been fruitful.

This new model was really an old one, mouse salmonellosis, but recast with more extensive and ramified biological dimensions so as to provide a phenotypic plasticity of the host population to nutritional manipulation of natural resistance. These new and extended dimensions were based on the operational utility, for these studies, of a genetically heterogeneous outbred host population infected with a polymorphic pathogen population.

Our experiments led to the discovery of a highly potent resistance factor. This factor is found in some natural foodstuffs. It is present there not innately but by virtue of an inevitable ecologic interaction of such foods, at their growth source, with microbial life. The newly discovered factor is neither a vitamin nor an antibiotic but may be biologically categorized as a member of a new class of ecological ectocrines, called by us the pacifarins, which furnish the chemical basis for mediation of the special interspecific, nonpredator relationships involved in infectious disease. The outcome of this mediation is the silent coexistence of host and pathogen, mice and *Salmonella* in the present model.

It has been possible to study the chemistry of the salmonellosis pacifarin, and highly purified preparations consistently reveal the features of an orthodihydric phenol conjugated with some amino acids, predominantly serine. A notable property is that of chelation, especially with ferric iron. Many other known iron-chelating substances, however, have no resistance-promoting properties, and the salmonellosis pacifarin remains unique in this respect. Multifactorial nutritional experiments reveal strong interactions with other classical nutrient entities, such as protein. And, very importantly, it has been shown that the avirulent salmonellae of the model biosynthesize a pacifarin, while a similar synthesis by virulent ones is undetectable. The macroworld of host mammalian nutrition and the microworld of pathogens have thus an important common feature in the pacifarins, and by advancing our understanding of it we may widen the biological base of our efforts to master infectious disease.

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26. A crude preparation of a serine containing iron chelate produced by a methionine-vitamin B_{12} auxotroph of *Escherichia coli* K_{12}, kindly supplied by Drs. N. Brot and H. Weissbach, proved inactive in our model.
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