the impersonal approach to the meaning and ramifications of life.

In my opinion, an opinion for which a surprising amount of support has been found, the time has come to end this constriction of horizons. Shortly, it may well become imperative to end it. I have particular reference to extraterrestial potentialities. Rocketeers have devoted untold hours of meditation and experimentation to their field, as have physicists, astronomers, and mathematicians. Although it is rather doubtful that astrogation charts will be immediately available, their preparation would be largely a matter of erudite compilation. Biologically, that far horizon is populated only with present-day hippogriffs and afreets, the bug-eyed monsters of science fiction. A more reliable segment of the earth's population should be represented on that frontier. Medicine alone has pushed slightly beyond the boundaries of the commonplace with studies of the effects on life of such influences as increased gravity or acceleration and the obverse, weightlessness. This advance has been timorous. The penetration should be both broader and deeper.

Who is to say that man is the ultimate of ultimates? If he is not, why have we not suffered visitations? Perhaps we have. It would require little imagination to justify a *cordon sanitaire* against such barbarians as us. Such thinking must, for the time being, remain moot, merely idle speculation. More to the point might be a reexamination of the indispensability of the opposable thumb to the development of intelligence. Even more to the point might be a consideration of the necessity of free oxygen to the development of superior forms, particularly since one is faced with known anaerobiosis and the theoretical possibility of a more radical redox. Would earthly temperature ranges be mandatory for even the theoretical silicon-based biochemistry? Should not the pioneer of the perhaps not-too-distant future be prepared, to some degree, not only for the vicissitudes of different time and gravity factors and atmospheres of varying degrees of tenability, but also for the eventuality of alien life, intelligent or otherwise? Should he not be supplied with the conclusions of the educated conjecturer, information of value even if little more than a point of departure? Might not the Martian canal(?) lichen(?) proliferate dangerously if offered warmth, air, and moisture? Or would such abundance prove toxic? What agents might control it, or what might be its metabolic properties?

I suggest that a field of intellectual endeavor, which might be termed "theoretical biologics," is worthy of the most serious consideration. Far from being facetious, I feel that the depth of understanding and the scope of knowledge that are required for effective, intelligent work in this field would preclude even the consideration of it by all but candidates for the doctorate and beyond. Unfortunately, many a recognized and respected Ph.D., by the very nature of his extreme specialization, could not boast of sufficient breadth. Perhaps no man ever could. Perhaps only cooperation and teamwork could effect any reasonable synthesis of ideas.

With the advent of the orbital unmanned satellite, perhaps some action will be taken on behalf of those who will form the crews in the next logical development. Certainly it would be stupid, criminally stupid, as well as wasteful of human life and treasure, to send men into space elegantly equipped with every device conceived by the physical scientists only to lose the entire expedition through the action of some biological agency that might have been anticipated. In the physical realm, as in the biological, eventualities beyond imagination might arise. Such could be borne with resolution. Within the scope of the imaginable, no matter how improbable, there can never be any excuse for the lack of foresight and preparation. To precisely such preparation, both experimentally and abstractly, the biologist might well direct his efforts, for, to borrow an expression, "It's later than you think."

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Dietary Casein Level and B-Factor Deficiencies Produced by Antagonists

The influence of dietary protein and carbohydrate levels on B-factor requirements has been given considerable attention. Among other things, it has been thought (*1, 2*) that the requirements are related to the need for B-factors in various enzyme systems in which it could be shown that they play an important part. In most previous studies, however, the presence of tissue stores of B-factors and their production by the intestinal flora interfered with the experiments. It therefore seemed desirable to re-examine some of the problems with the help of reliable antivitamins, which are now available.

Weanling albino rats from a uniform colony were distributed into matching groups of eight to 16 animals. They were fed highly purified diets that contained dextrose and casein in varying ratios and 10 percent lard, together with cellulose, salts, and liberal amounts of all vitamins (*3*). The casein had been made virtually vitamin-free by a special method of purification. All procedures have been previously described in detail (*2*).

When thiamine-free diets containing 5, 30, or 84 percent casein along with 79, 54, or 0 percent dextrose were used, the rats that received the lowest level of protein died earlier than those that received the higher ones; this is in agreement with the observations that replacement of carbohydrate by protein (*3*) or fats (*4*) has a thiamine-sparing effect. It is usually believed (*5*) that this is the result of the fact that thiamine is chiefly involved in the enzyme systems necessary for the metabolism of carbohydrate rather than the systems necessary for the metabolism of protein and fat.

However, when rats were placed on the same diets and injected daily with 50 micrograms of pyrithiamine (the most potent thiamine antagonist, *6*) they died, on the average, much earlier than those that had not received the antivitamin. Moreover, in contrast to the uninjected groups, those on the lowest protein intake survived significantly longer than those on the higher levels (Fig. 1). These experiments were made twice (with groups of eight and 16 rats), and both times the differences observed were found to be statistically significant.

On the basis of this experiment, it can be concluded that the thiamine requirements of rats increase with increasing dietary protein levels if the rigid exclusion of thiamine from the diet is associated with the use of pyrithiamine, which denies the animals the use of both thiamine stores and that produced by the intestinal flora. If these requirements have any relationship to the role of thiamine in enzyme systems and if pyrithiamine exerts no effect other than that of a thiamine antagonist, one must conclude that thiamine plays an important part not only in carbohydrate metabolism but also in protein metabolism.

It has been demonstrated (*2*) that the riboflavin requirements of rats fed the potent riboflavin antagonist, galactoflavin, and maintained on riboflavin-deficient diets increase with increasing dietary protein levels. This could be concluded from observations on survival rates, body weights, and food consumptions. This and other considerations strongly indicated that protein and riboflavin are mutually limiting factors in metabolism.

Increased pyridoxine requirements with increasing dietary protein levels have been found even when no antagonist was administered (*7*). In our experiments, the animals were fed pyridoxine-deficient diets containing 5, 30, or 74 percent casein and were given daily oral feedings of the pyridoxine antagonist, desoxypyri-

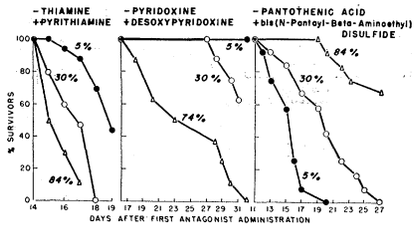

Fig. 1. Survival rate of B-factor-deficient rats on purified diets with varying casein levels and supplied with the appropriate antivitamin.

doxine; observations were made of growth, food intake, development of the "acute deficiency" state (*8*), and survival time. In all these aspects, the animals on 74-percent casein showed the most severe changes; those receiving 5 percent showed scarcely any and the ones fed 30 percent were in between. Death, in particular, occurred earliest with 74-percent casein and not at all during the time of observation among those on 5-percent casein (Fig. 1). Thus, the use of an antagonist does not alter, but strongly intensifies, the differences in pyridoxine requirements, which go parallel to the casein content of the diet. The rapid occurrence of a severe deficiency state at high dietary protein levels is indicative of the great importance of pyridoxine in the protein-metabolizing enzyme systems. As in the case of riboflavin and thiamine, pyridoxine and protein are mutually limiting factors.

It has been found that the signs of pantothenic acid deficiency become less pronounced if the carbohydrate in the diet is partly replaced by protein (*9*); no antagonists were used in those experiments. In our experiments, groups of 12 rats received 5,- 30-, and 84-percent casein in pantothenic acid-deficient diets and daily injections of 10 milligrams of the pantothenic acid antagonist, bis(*N*-pantoyl-beta-aminoethyl) disulfide (*10*). In Fig. 1 it is demonstrated that the survival rate of these animals increased very significantly when carbohydrate in the diet was replaced by protein. Although the casein had been specially purified, these experiments (just as previous ones) did not entirely rule out the possibility that the milder form of the deficiency state was the result of the presence of traces of pantothenic acid in the casein. This argument could be entirely refuted by an experiment in which eight animals that received 30-percent casein were restricted in their food intake so that they just maintained their body weights. This group survived the freely eating, slowly growing, deficient animals on the same diet. Therefore, the longer life span of animals on the high casein level is not the result of traces of pantothenic acid in the casein because the animals on the restricted food intake that survived their controls received less casein. The experiment further demonstrates that the signs of the deficiency state are less pronounced if the food intake is restricted.

Despite the rigid exclusion of pantothenic acid from the diet and the use of a potent antagonist, increased dietary protein levels decrease the pantothenate requirements; thus, pantothenic acid and protein are probably not mutually limiting factors. Inasmuch as, in these experiments, high dietary protein levels mean low carbohydrate levels and vice versa, these experiments indicate that such mutual limitation exists between carbohydrate and pantothenate.

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Regeneration of X-rayed Salamander Limbs Provided with Normal Epidermis

It has been suggested that a large part of the regenerate of a salamander limb arises from epidermal cells (*1, 2*). The evidence for this is that, as the blastema forms, approximately the same number of mesenchymal regeneration cells appear as there are epidermal cells disappearing. It is believed that the epidermal cells change into mesenchymal cells *in situ* in the epidermal cap. This transformation had been described earlier (*3*) but had not been interpreted as evidence for a permanent change. Other investigators, while confirming the loss of many epidermal cells during blastema formation (*4, 5*), believe that these cells die, but this has not been studied quantitatively. Still others, for various indirect reasons, believe that a transformation from differeniated epidermis to a variety of internal limb tissues is unlikely (*6–10*). Direct evidence that epidermal cells can transform to mesenchymal cells was obtained by tracing polyploid epidermis on a diploid limb stump and later recovering it as mesenchymal tissue (*11*). There is still a question whether the marked mesenchymal cells that had been epidermal could have completed the transformation to differentiated mesodermal tissue.

Because of general interest in the extent of cellular transformation during regeneration and because there have been different interpretations of data already presented, a more rigorous test of the possibility of epidermal transformation has been made.

The best method for testing the potency of a tissue during limb regeneration is the method of transplanting it to an x-rayed limb that is incapable of growth or regeneration. Sufficient x-irradiation completely prevents growth and regeneration. The change appears to be permanent and irreversible (*12*). If normal tissue is transplanted to an x-rayed limb and the limb is subsequently amputated at the level of the graft, whatever regenerates must arise from the grafted tissue. The method has been used previously to demonstrate that bones, muscles, and whole skin can each produce whole limbs (*13, 14*). In the present work (*15*), an attempt has been made to learn whether the epidermis alone can furnish the cells for limb regeneration.

Both forelimbs of 55 adult *Triturus viridescens* were x-rayed below the elbow with single dosages of 500 to 10,000 roentgens. The conditions of irradiation were 150 kv and 8 ma with a delivery rate of 745 r per minute at a distance of 11.5 cm. The upper arms and the rest of the animal were protected by lead shields. Left forelimbs served as controls. These were irradiated and either 7 or 28 days later were amputated midway between wrist and elbow. Right forelimbs were treated in the same way except that, in addition, whole skin was stripped off the stump up to the unirradiated tissue 1 mm above the elbow at the time of amputation. It was known from work with polyploid skin transplants that only the epidermis migrates and piles up at the tip of the stump (*11*). Hence, in the right limbs, the epidermis that migrates to cover the stumps was unirradiated.

On all 55 animals the limbs that became covered with unirradiated epidermis regenerated. No complete hands regenerated from the control stumps. After the 3 lowest x-ray dosages, 500, 1000, and 2000 r, there was some outgrowth