commission. Up to date, the total of such appropriations has been slightly over $100,000. This continued support over a period of at least 18 years, during much of which time the income of the Chemical Foundation has been steadily decreasing, is evidence of the interest of that body in the work of the Stain Commission. Biologists who feel that this work has been of any value in assuring them reliable stains must acknowledge a decided debt of gratitude to the Chemical Foundation and to its president, the late Mr. Francis P. Garvin.

Up to 1931 the entire cost of administering the Stain Commission was borne by the foundation. By this time, however, the commission was beginning to earn some income of its own: Its publication work, which had been self-supporting from the beginning, was by that time realizing a small profit, and an appreciable income was being received from the stain companies through fees charged for testing their products and through the sale of certification labels. Inasmuch as the income of the Chemical Foundation, derived chiefly from royalties on patents, was then decreasing, it was decided that the commission must be made as nearly self-supporting as possible. As a result the foundation's appropriations were progressively decreased, and all possible efforts were made to increase the commission's earned income.

By 1933 the appropriations had been cut down to $3,200 a year and have remained approximately at that figure ever since. The balance was at first made up partly by current earned income and partly by using publication profits which had been put aside during the more prosperous years. During the last two or three years current earned income together with the foundation's contribution has been sufficient. The latter has amounted to only about 45 per cent. of the entire budget. Although efforts have continually been made to increase the earned income, it has not yet become sufficient to balance the budget completely. In a sense the stain testing is self-supporting, for it yields sufficient income to pay for all the time actually spent on routine testing. Unfortunately, however, the rest of the time of the assistants doing the work must be paid for, and the present earned income does not cover that. Since the balance of their time is largely spent on investigation, it can be said that it is only the research of the Stain Commission that must be supported by some outside organization. As the last year has seen a noticeable increase in the earned income, probably $2,000 annually from such outside source would now be sufficient.

At present, when such is the financial situation of the commission, the support of the Chemical Foundation must be entirely withdrawn. This comes about through no lack of interest on the part of the officers of the foundation, but merely because its patents are expiring and its income accordingly diminishing rapidly. It raises the question, however, whether the work of the Stain Commission must be stopped or so seriously curtailed as to destroy its efficiency, merely because of the lack of the comparatively small sum of money mentioned above.

This matter is now called to the attention of biologists. The executive committee feels that the work of the commission is of some use to biologists and that it would be unfortunate if it should have to stop or to be badly crippled when it lacks so little of being self-supporting and its need is probably only temporary. If any users of stains feel similarly on the subject and have any practical suggestions to make, communications from them in regard to the matter would be greatly appreciated.

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SPECIAL ARTICLES

THE LOSS OF RESISTANCE TO MURINE TYPHUS INFECTION RESULTING FROM RIBOFLAVIN DEFICIENCY IN RATS[1]

FROM observations on the intracellular behavior of typhus rickettsiae at various temperatures in plasma tissue cultures and in Maitland cultures, one of us[2] concluded that the unrestricted multiplication of these organisms, resulting in distention of infected cells, might be dependent upon a lowering of intracellular metabolic activity.

In view of the fact that the essential feature of riboflavin deficiency is believed to be interference with intracellular oxidation processes[3] it seemed, on theoretical grounds, that the susceptibility of experimental animals to typhus infection might be greatly enhanced by this type of dietary deficiency. The preliminary

[1] From the Department of Pathology, Harvard Medical School, and the Harvard Dental School, Boston, Massachusetts.

[2] Henry Pinkerton, *Arch. Exp. Zellforsch.*, 15: 425, 1934.

[3] A. G. Hogan, *Jour. Am. Med. Assn.*, 110: 1188, 1938.

experiment to be reported here strongly supports this hypothesis.

METHOD OF PRODUCTION AND COURSE OF THE DIETARY DEFICIENCY STUDIED

Albino rats (Wistar strain) weighing 40 to 50 grams, from a stock kept on Purina dog chow were placed in individual cages with raised screen bottoms ($\frac{1}{2}$-inch mesh) and given *ad libitum* water and the following diet:

	Per cent.
Casein (flavin-free)[4]	18
Salt mixture[5]	4
Dextrose	32
Corn starch	31
Rice polishing concentrate[6]	6
Lard	5
Cod liver oil	4

Growth ceases after the third week on this diet. Sore and swollen eyelids and a definite keratitis appear after 6 to 8 weeks, followed by clouding and ulceration of the cornea.[7] Irregular loss of hair occurs on the face, shoulders and back. The skin of the extremities becomes dry, rough and often swollen and ulcerated. Animals survive on this diet for from 10 to 14 weeks, and show all the typical symptoms of riboflavin deficiency.

EXPERIMENTAL OBSERVATIONS

One rat showing advanced symptoms of the deficiency, one moderately advanced, and three showing early symptoms (after only 7 weeks on the above diet) were injected intraperitoneally with Mexican typhus virus. The inoculum used for the first two rats contained large numbers of rickettsiae, while that used for the last three rats contained relatively small numbers of organisms. For controls, 2 rats in an advanced stage of vitamin A deficiency were injected with large doses of rickettsiae, and three rats on a normal diet with relatively small doses.

The five riboflavin-deficient rats all died on the fourth, fifth and sixth days after injection. The peritoneal surfaces of all five of these rats were bathed with a mucinous exudate, sufficient in amount to allow the accumulation of about 0.5 cc from each by scraping lightly with a dull knife. Smears of the exudate from each case showed large clumps of serosal cells distended with rickettsiae in every low-power field. In many preparations practically every serosal cell was distended. This picture was found in female rats as well as in male rats. The distention of these cells was often so great that the nuclei were compressed into small hyperchromatic spherical masses. The exudate also contained many polymorphonuclear leucocytes, which were often heavily laden with rickettsiae, and many extracellular rickettsiae, probably escaped from ruptured serosal cells. In at least four of these rats, death was undoubtedly due to typhus infection, since uninjected rats in the same group survived them by several weeks.

One of the vitamin A deficient rats died on the sixth day after injection, at a time when death from the deficiency alone was anticipated. The peritoneal surfaces showed no grossly demonstrable exudate. Moderate numbers of extracellular rickettsiae were found in smears made by scraping the peritoneal surfaces of this rat. Very few rickettsia-laden cells were found, however, and no distended cells comparable to those which were seen in great numbers in the riboflavin-deficient rats. In view of the large original dose, it seemed possible that many of the organisms seen may have been survivors from those originally injected. The other vitamin A deficient rat was killed on the eighth day after injection. The peritoneal surfaces were normal and no rickettsiae were found in smears. One of the three rats on normal diet was chosen arbitrarily and killed for study on the fifth day after injection. Here again there was no peritoneal exudate. A few extracellular rickettsiae and a rare infected cell were found in smears from the peritoneum of this rat. The other two rats on a normal diet showed no external evidence of infection and were allowed to recover. It is well known that endemic typhus is never a fatal disease in normal rats.

Regaud-fixed Giemsa-stained sections of the tissues of three of the above riboflavin-deficient rats were studied. These sections furnished additional evidence of the markedly lowered resistance of these animals to the rickettsial infection. Not only were the majority of the serosal cells found to be laden with rickettsiae, but the endothelial cells in several organs were similarly invaded. In many areas, for example, every third or fourth Kupfer cell in the liver was greatly distended with these organisms. Rickettsiae have not previously been demonstrated in the Kupfer cells of experimental typhus animals under any conditions.

DISCUSSION

The above observations are believed to be of interest for several reasons. The technique described may well prove to be an important supplement to the methods now in use for the production of vaccines in rickettsial diseases. It is also possible that the method may be used advantageously in the study of filtrable viruses,

[4] Extracted twice over night with 60 per cent. alcohol and twice by refluxing for two hours with 95 per cent. alcohol.

[5] U. S. Pharmacopoeia XI.

[6] Borden Company, New York.

[7] O. A. Bessey and S. B. Wolbach, *Jour. Exp. Med.*, 69: 1, 1, 1939.

especially those which have a relatively low virulence for experimental animals under normal conditions. Studies of this type of deficiency in experimental animals of other species are in progress.

SUMMARY

Riboflavin deficiency, even in a relatively early stage, greatly lowers the resistance of the rat to endemic typhus, thereby resulting in a fatal disease. Not only the serosal cells, but also the endothelial cells in several organs, notably the Kupfer cells of the liver, become greatly distended with rickettsiae under these conditions. Preliminary experiments strongly suggest that advanced vitamin A deficiency, even when making animals markedly cachetic, does not have a comparable effect. Riboflavin deficient animals remain alive for several weeks with an abnormal intracellular metabolism. This type of deficiency is worthy of further study as a possible method of approach in the investigation of other intracellular parasites and filtrable viruses. Speculation regarding the mechanism concerned also suggests the desirability of a study of the effects of this deficiency upon the production of immune bodies in general.

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PRELIMINARY NOTE ON THE MODE OF UNION OF THE GALACTURONIC RESIDUES IN PECTIC ACID

CONSIDERABLE progress has been made in recent years in the field of the chemistry of pectic acid, particularly through the investigations of Link and his co-workers. However, the essential points in its structure are still missing, namely, the exact position of the linkage of the individual residues of the galacturonic acid, their ring structure and the length of the chain (if pectic acid has a straight chain structure). Levene and Kreider recently limited the place of union of the individual residues to positions (4) or (5). The choice between the two has not yet been made.

For the purpose of obtaining the missing information, pectic acid has been exhaustively methylated. The product thus obtained had a composition corresponding most satisfactorily to a structure composed of approximately six units.

$C_{56}H_{90}O_{37}$.	Calculated.	OCH_3, 45.80
	Found.	OCH_3, 45.40

On saponification the substance had the following methoxyl value:

$C_{50}H_{78}O_{37}$.	Calculated.	OCH_3, 34.18
	Found.	OCH_3, 34.30

Whether the material is only a methylated fragment of pectic acid can as yet not be stated. The deduction as to the length of the chain of this material is tentative, inasmuch as it is based only on the methoxyl value in the fully methylated and in the saponified material. More precise information on this point is expected from the analysis of the methylated polygalactoside, obtained on reduction of the above material. This substance has now been prepared by heating the exhaustively methylated material with copper chromite catalyst in an atmosphere of hydrogen at a temperature of 175° and a pressure of 3,500 pounds per square inch during 6 hours. The product has been obtained in only a fair degree of purity, having the following composition:

$C_{56}H_{102}O_{31}$.	Calculated.	C 52.9, H 8.1, OCH_3, 48.81
	Found.	C 53.8, H 8.0, OCH_3, 47.33

As far as can be judged from the rate of hydrolysis of the fully methylated pectic acid, the galacturonic residues seem to have a furanose structure, for after 2½ hours heating of the product with 0.01 *N* hydrochloric acid, at 100° C., about 1½ equivalents of reducing groups are developed and after 15 hours, about 3 equivalents. The methyl ester of 2,3,4-trimethyl α-methyl-d-galacturonide under identical conditions remained unchanged. (R. S. Tipson.)

Thus, it seems suggestive that the galacturonic acid residues of pectic acid have a furanose structure and hence the union of the individual residues is through carbon atom (5).

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VITAMIN E AND NUTRITIONAL MUSCULAR DYSTROPHY

IN 1931 Goettsch and Pappenheimer[1] described a dietary deficiency disease in rabbits and guinea pigs characterized by dystrophy of the voluntary muscles. Morgulis and Spencer,[2] Morgulis, Wilder and Eppstein,[3] found that at least two factors, both contained in whole wheat germ, were required for the prevention or cure of the disease. One factor was removed from wheat germ, previously extracted with petroleum ether, by 70 per cent. ethanol. The other factor was found in the unsaponifiable fraction of wheat germ oil. This suggested that the fat-soluble factor might be vitamin E. However, the dystrophy producing diet apparently contains a significant amount of vitamin E, since both Goettsch and Pappenheimer[1] and Morgulis[4] found

[1] M. Goettsch and A. M. Pappenheimer, *Jour. Exp. Med.*, 54: 145, 1931.
[2] S. Morgulis and H. C. Spencer, *Jour. Nutrition*, 11: 573, 1936.
[3] S. Morgulis, V. M. Wilder and S. H. Eppstein, *Jour. Nutrition*, 16: 219, 1938.
[4] S. Morgulis, "Nutritional Muscular Dystrophy," Hermann and Cie., Paris, 1938.