FURTHER STUDIES ON ORAL IMMUNIZATION TO COLDS

IN a previous report[1] we gave our results obtained during the winter of 1933–34, in the prevention of colds by oral administration of a cold vaccine which was high in heterophile antigen. In that work, 462 persons who took the vaccine orally showed a decrease of 57 per cent. in the incidence of colds, whereas 527 controls showed a decrease of only 12 per cent. This amounts essentially to a decrease of 45 per cent. due to the oral vaccine.

This last winter, 1934–35, we have given the oral cold vaccine to 445 persons and observed 469 others as controls. The results are shown in the table.

RESULT OF STUDIES OF IMMUNIZATION TO COLDS BY ORAL VACCINE FOR WINTER OF 1934–35

	W. S. Life Ins. employees		Medical students		U. C. Life Ins. employees		General group		Nurses		Total	
	Took vac.	Controls	Took vac.	Controls	Took vac.	Controls	Took vac.	Controls	Took vac.	Controls	Took vac.	Controls
*No. cases	87	100	80	87	31	48	171	201	30	33	399	469
Average total number colds per year in past three years	291	176	296	264	98	77	768	555	101	64	1554	1136
Total number colds experimental year ..	97	119	87	223	52	59	185	385	44	51	465	837
Decrease in number of colds ..	194	57	209	41	46	18	583	170	57	13	1089	299
Per cent. decreased	66.6	31.2	70.6	15.5	46.9	23.4	75.9	30.6	56.4	20.3	70.0	26.3

* Forty-six cases who reported continuous colds in previous years, although taking the vaccine, this year were not included in the figures of this table. These forty-six cases can be summarized as follows; three were not helped, they still had continuous colds; forty-three stated they were greatly benefited, reporting a total of thirty-four colds this winter for the forty-three individuals.

Ninety-four cases who started the vaccine but only took the vaccine for a few days or several weeks are not included in this figure.

Although all heterophile antigens have similar properties, there is sufficient evidence to indicate that homologous heterophile antigens and antibodies have a greater affinity for each other than heterologous[2] ones. Hence the oral heterophile cold vaccine which started as a combination of the pneumococcus DR I, and streptococcus has been improved by the addition of other bacteria which infect the respiratory tract. The strains were selected for their heterophile content and their ability to resist the effects of the gastro-intestinal secretions. Additional strains of pneumococci were also used. The bacterial cultures were sterilized, the bacteria separated, absorbed on starch, dried and finally filled into capsules. Each capsule contained pneumococci 25 billion, *H. influenza* 5 billion, streptococci 15 billion and *M. catarrhalis* 5 billion.

It is necessary that the vaccine be taken with cold water on an empty stomach, as otherwise the action of the stomach juices on the vaccine prevents the absorption of the complete antigen. Frequent administration of the vaccine is also essential, since the resultant immunity is of very short duration.[3] For these reasons the treatment consisted of the ingestion of one capsule with a half glass of cold water at least one half hour before breakfast for seven consecutive mornings, after which one to two capsules per week were taken throughout the season.

Reactions following the use of the oral vaccine occurred occasionally, and these were usually in persons who started taking the vaccine just after recovering from a cold. The reaction manifested itself as a grippy feeling or a mild intestinal discomfort.

SUMMARY

(1) Among the 445 persons taking the vaccine, 399 had 1,089 (70 per cent.) less colds this year than usual, whereas the 469 controls showed a decrease of only 299 (26.3 per cent.) colds, an essential decrease of 43.7 per cent. due to oral cold vaccine.

(2) It was stated by 326 (81.7 per cent.) of the 399 persons taking the vaccine that they had been greatly benefited.

(3) Of forty-six persons who had previously been troubled with practically continuous colds, three reported that they were not helped while taking the vaccine, while the remaining forty-three, suffering a total of only thirty-four colds during this winter, were

1 George E. Rockwell, Herman C. Van Kirk and H. M. Powell, *Jour. Immunol.*, 28: 475, 1935.

2 C. H. Bailey and M. S. Shorb, *Amer. Jour. Hyg.*, 17: 358, 1935; R. Landsteiner and P. Levine, *Jour. Immunol.*, 22: 75, 1932.

3 George E. Rockwell and Herman C. Van Kirk, *Jour. Bacteriology*, 29: 47, 1935.

greatly benefited. In other words, these forty-three persons, previously in a state of practically continuous coryza, suffered from less than one cold per person during this season.

(4) The oral vaccine makes frequent administration practical, which is so essential in such short life immunities as occur with the bacteria of upper respiratory infections.

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A "GROWTH SUBSTANCE"

IN connection with a survey of the nutritional requirements of pathogenic yeasts and molds being carried on in this laboratory in collaboration with Dr. L. B. Kingery, of the Department of Dermatology, University of Oregon Medical School, we observed the case of an organism isolated from a deep skin lesion, which the authors decided to investigate more fully.

The organism grows with extreme rapidity in a suitable medium, is easily suspended and handled and is reasonably uniform in its responses. Aside from the ordinary nutrients used for yeast culture[1] this organism requires an assortment of amino-acids and is stimulated appreciably both by pantothenic acid and crystalline vitamin B. To promote rapid growth, however, there is required in addition another water-soluble substance which is particularly abundant in liver, liver extract No. 343 (Eli Lilly), kale and alfalfa, and to a lesser but considerable extent in milk. Its effect is striking in the extreme and becomes apparent two or three hours after seeding with the organism.

Since liver and kale, the richest known sources, have remarkable nutritive properties for higher animals, the latter being of extraordinary value for poultry and dairy cows, we suspect that the "growth substance" under consideration is of importance in the nutrition of animals as well as of the organism which we are using as a tool.

This nutrilite has a tendency to be associated with proteins, such as casein and egg albumin, to such an extent that for a considerable period of time we worked upon the supposition that the substance was an unknown amino-acid such as is being investigated by Rose[2] and his co-workers. It was later found, however, that careful purification of casein previous to hydrolysis removes all but traces of the stimulating substance.

Concentration of the "growth factor" has been accomplished to such an extent that one part of the preparation gives a response when added to ten million parts of culture medium. The preliminary steps in the concentration are the same as are used by Kuhn[3] in the preparation of lactoflavin. That our growth factor is not lactoflavin itself is shown by the fact that its physiological effect is practically unimpaired by long exposure to light, whereas under the conditions used the lactoflavin present in the preparation was largely decolorized and destroyed. Also through the kind courtesy of Dr. Kuhn, of the Kaiser-Wilhelm Institut in Heidelberg, we have been furnished a sample of pure crystalline lactoflavin which gives negative results when used as a substitute for our cruder material. We can not be sure, however, that pure lactoflavin is not *one* factor concerned in the response.

It appears that in the later steps of the purification of lactoflavin, material is discarded which is physiologically very active (at least toward certain lower organisms). This interpretation is in line with the recent work of Booher[4] who finds that what may be regarded as a crude lactoflavin preparation is effective for experimental rats, under conditions where Kuhn finds the pure pigment to fail entirely.

Our results would call in question the usefulness of such partially refined lactoflavin preparations as that of Itter, Orent and McCollum,[5] which may or may not owe its physiological action to lactoflavin itself. It should be noted that from 10 pounds of whey powder these workers recovered only what is equivalent to about 700 units of "vitamin G." This can not be more than a one or two per cent. yield on the basis of that present in the original whey powder. This fact in itself indicates that something of outstanding importance has been discarded during the purification.

The nutrilite with which we are concerned is very stable; it is only partially destroyed by long autoclaving at 20 pounds pressure in 3N (or even 5N) H_2SO_4 solution, or in 2N $Ba(OH)_2$ solution. The concentration of this substance is being continued; it seems likely that the microorganism affected may be a valuable tool in helping to clear up the confusion regarding the number and chemical characteristics of the water soluble vitamins which remain as yet unknown.

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[1] Williams and Saunders, *Biochem. Jour.*, 28: 1887, 1934.

[2] Rose, *Proc. Amer. Soc. Biol. Chem.*, 8: 63, 1934.

[3] Kuhn, Gyorgy and Wagner-Jauregg, *Ber. der Deuts. Chem. Gesell.*, 66B: 1037, 1933.

[4] Booher, *Jour. Biol. Chem.*, 107: 591, 1934.

[5] Itter, Orent and McCollum, *Jour. Biol. Chem.*, 108: 579, 1935.