produced growth of *S. cerevisiae*, either in the presence or in the absence of 5-fluorouracil. The ability of myoinositol to promote growth of *S. cerevisiae* is well known[4].

When the concentration of 5-fluorouracil was increased in the culture medium from the usual level of $7 \cdot 10^{-6}$ to $10^{-4} M$, myoinositol[5] no longer was effective in restoring growth. Cytosine[5] and uracil[5] were both active under these conditions, with the former significantly more active than the latter. These results in yeast may be related to the role of cytidine diphosphate in the biosynthesis of myoinositol phosphatides in the mammal[6].

To our knowledge, this is the first report indicating a higher excretion of myoinositol by the new-born infant than by adults. Although work has been done on adult excretion of myoinositol[7–12], the only report dealing with children is the early observation of KULZ[13]. He concludes that the urine of adults and children with a wide variety of diseases may contain myoinositol. It has been shown that the myoinositol content of fetal blood and fetal fluids is consistently higher than in the mother[14].

Zusammenfassung. Ein aktives Agens im Säuglingsurin, das die Hemmung von *Saccharomyces cerevisiae* durch 5-Fluorouracil verhindert, wurde als Myoinositol identifiziert und generell bei allen Neugeborenen, ebenfalls bei einer Anzahl von Kindern mit allgemeiner Aminoacidurie, gefunden. Gesunde Erwachsene zeigten keine nennenswerten Mengen.

J. P. SCHIRMER JR., H. TIECKELMANN, and R. GUTHRIE

Department of Chemistry and Department of Pediatrics, State University of New York at Buffalo (New York USA), July 27, 1964.

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Studies on the Chemical Composition of Streptococcal Co-opsonin

It has been reported recently that absorption of human serum with bentonite results in removal of a non-specific 'co-opsonin', which is necessary for optimal phagocytosis of virulent group A streptococci by polymorphonuclear leucocytes in the presence of type-specific antibodies[1,2]. Amounts of bentonite which almost completely inactivated the serum opsonic activity did not reduce significantly either complement, properdin, or any of the major components of the complement system. This paper describes attempts to characterize the chemical nature of this co-opsonin. It will be shown that it is distinct from lysozyme, and that it may reside in the serum β-lipoprotein moiety.

Materials and methods. Removal of the co-opsonin: Fresh serum from the same donor (M.W.R.) collected in a fasting state, was used in all experiments. The procedure of co-opsonin absorption has been described in detail in a previous communication[2] and will be summarized only briefly. Serum was incubated at 37°C for 20 min with the desired amounts of bentonite. It was then separated by centrifugation (6800 × *g* for 30 min at 4°C in a Model-L Spinco ultracentrifuge with a No. 30 rotor head) and stored at −70°C until ready for use. Stock suspensions of bentonite were prepared by adding the dry powder (Bentonite, U.S.P., Fischer, Lot No. 790219) to distilled water in a concentration of 3 mg per ml. Particle dispersion was improved by mixing the suspension in a Waring blendor for 1 min. Bentonite in amounts desired for serum absorption was separated from stock suspension by centrifugation (2800 × *g* for 30 min).

The phagocytic test: The phagocytic test ('Bactericidal Test') was identical with that employed in previous studies[1,2]. Overnight Todd-Hewitt broth cultures of a strain of type 6 group A streptococcus (T-6/543) adjusted to the desired concentration, type-specific rabbit antiserum, fresh human serum (M.W.R.) as source of natural co-opsonins, and autologous polymorphonuclear leucocytes, twice washed in physiologic saline, were employed in all experiments. The degree of phagocytosis was expressed by the 'bactericidal index' (BI), which is determined by the relative inhibition of the growth of the streptococcus in the phagocytic test; i.e.

$$\text{Bactericidal index} = \frac{\text{No. of organisms in the inoculum}}{\text{No. of organisms surviving phagocytosis in the presence of type-specific antibody and test serum}} \times \text{Growth in absence of type-specific antibody}$$

As is apparent, the greater the degree of destruction of streptococci by phagocytosis, the greater the numerical value of the bactericidal index.

Complement titration: Complement titration was carried out to the 50% hemolytic endpoint according to the method of KABAT and MAYER[3].

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Properdin titration: The zymosan (Fleischman Lab., Lot No. OB 298) assay, as described by KENT et al.[4], was utilized.

Lysozyme determination: The lysozyme determination was based on that employed by MYRVIK and WEISER[5]. Commercial reagents, consisting of crystalline egg-white lysozyme (Bacto-lysozyme, Difco, Lot No. 465), and ultraviolet-killed, dried culture of *Micrococcus lysodeikticus* (Bacto-lysozyme substrate, Difco, Lot No. 461-10) were used throughout.

Separation of β-lipoproteins: Density gradient ultracentrifugation was employed. The density of the serum specimen was adjusted to 1.063 by the addition of 28% of potassium bromide. This solution was then centrifuged for 20 h at 105,500 × *g* in Model-L Spinco ultracentrifuge, in a No. 30 rotor head, at 4°C. The top 1 ml containing all lipoproteins with densities less than 1.063 g/ml was carefully separated. The resultant two fractions were dialyzed in distilled water for 24 h in the cold. The fractions were then concentrated in polyvinyl pyrrolidine to their original volumes.

Determination of β-lipoproteins: A quantitative immunoprecipitation method employing specific anti-β-lipoprotein antibodies (β-L Test, Hyland Lab., Lot No. 371 ITI), was utilized[6]. One drop of test serum was mixed with 2 drops of antiserum, and the resultant mixture drawn into a capillary tube whose one end was then sealed by flame. After a 15 min incubation at room temperature, the capillary tubes were centrifuged for 10 min. The results were expressed in terms of the 'immunocrit', which was derived from the following relationship:

$$\text{Immunocrit} = \frac{\text{length of column of precipitate}}{\text{length of column of precipitate and supernatant}} \times 100.$$

The average immunocrit in 30 healthy young males was found to be 4.11 with a standard deviation of ± 0.42.

Electrophoresis: Standard paper-strip electrophoretic procedures utilizing Spinco equipment and reagents were employed[7].

Results. Absorptions of serum with bentonite resulted in an almost complete removal of streptococcal co-opsonins (Table). Even when as little as 0.5 mg of bentonite per ml of serum was used, the bactericidal index diminished from 1240 to 6. There was no significant decrease in the levels of complement or properdin with this amount of bentonite. The β-lipoprotein levels, however, were significantly decreased, and lysozyme activity was completely removed. When amounts of bentonite, in excess of 3 mg, were employed for absorption, complement and properdin could no longer be detected (Table).

Because lysozyme and β-lipoproteins were two of the serum constituents (in addition to the co-opsonin) that were reduced or removed by minute amounts of bentonite, their role in streptococcal phagocytosis was investigated further.

When β-lipoproteins were removed completely from the serum by density gradient ultracentrifugation, the bactericidal index diminished from 2257 to 5. However, recombination of bentonite-absorbed serum with the ultracentrifugal β-lipoprotein fraction did not restore co-opsonin activity of the serum. Addition of purified lysozyme in normal serum concentrations to the bentonite-absorbed serum, similarly failed to re-establish the co-opsonin activity.

Comments. This study confirmed the previously reported selective absorption by bentonite of serum β-lipoproteins[8], lysozyme[9], as well as streptococcal co-opsonin[1,2]. Because of this similar affinity for bentonite, it was considered possible that the co-opsonin activity of the serum may have resided in the β-lipoprotein moiety, or may have been associated with lysozyme.

The reasons for the failure to restore opsonic activity of bentonite-adsorbed serum with purified β-lipoproteins have not been ascertained. Co-opsonins could be extremely labile so that relatively gentle procedures, as have been employed here for the β-lipoprotein isolation, could result in irreversible inactivation. Moreover, other factors distinct from β-lipoproteins, but participating in the opsonic reaction, may have been removed by the bentonite absorption.

Lysozyme has been shown recently to possess a wide antibacterial spectrum when acting in conjunction with specific antibodies and complement[10]. Because both antibodies and complement are present under the test conditions of streptococcal phagocytosis, it was thought that lysozyme could participate in the bactericidal reaction, either as the 'co-opsonin' or (more likely) directly as a bacteriolysin. However, restoration of lysozyme activity

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Effect of serum absorption with bentonite on the levels of different factors

Serum treated with bentonite[a]	Bactericidal index	Complement ($C'H_{50}$ u.)	Properdin (units)	Lysozyme (mcg)	β-lipo-proteins[b] (immunocrit)	γ-globulin %	β-globulin %
Unabsorbed serum	1240	40.9	8	6.8	4.48	10.5	16.6
0.5	6	37.9	8	0	3.51	10.9	15.5
1.5	6	33.6	8	0	3.40	9.8	14.1
3.0	5	0	0	0	3.16	9.1	13.2
6.0	5	0	0	0	2.95	8.6	13.1
12.0	3	0	0	0	2.55	8.9	12.8
24.0	4	0	0	0	1.68	7.2	12.8

[a] mg of bentonite per ml of (M.W.R.) serum. [b] Average of 3 determinations

to bentonite-absorbed serum, failed to increase the bactericidal index[11,12].

Zusammenfassung. Das natürliche an Bentonit adsorbierbare «Co-opsonin», welches für eine optimale Phagocytose virulenter Streptokokken der Gruppe A nötig ist, erweist sich als verschieden von Lysozym. Es liegen Hinweise vor, dass das «Co-opsonin» in der β-Lipoprotein-Fraktion des Serums vorliegt.

M. W. Rytel, R. I. Lytle, and G. A. Sanders

Division of Biochemistry, Naval Medical Research Unit No. 4, U.S. Naval Hospital, Great Lakes (Illinois USA), June 15, 1964.

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[12] From Research Project MR 005.12-1102, Bureau of Medicine and Surgery, Navy Department, Washington (D.C. USA). The opinions expressed herein are those of the authors and cannot be construed as reflecting the views of the Navy Department or of the Naval Service at large. Usage of commercially available materials cannot be construed as implying endorsement or preference for those products over other similar products available on the market. Requests for reprints should be addressed to: Officer in Charge, Naval Medical Research Unit No. 4, U.S. Naval Hospital, Great Lakes (Illinois USA).

Reaction of 6-Aminopenicillanic Acid with Frequentin

When 6-aminopenicillanic acid (6-APA) (I) was added to fungal fermentations it was possible to detect the occurrence of at least three different types of reaction other than those associated with penicillinase activity[1], its reactivity with other molecules of 6-APA[2,3] or with CO_2[4–6]. The first was found to be independent of the nature of the micro-organism and was in fact, shown to be the reaction of 6-APA with reducing sugars[7]. The second reaction is the synthesis of penicillins from added 6-APA by micro-organisms which do not necessarily produce penicillins in the absence of 6-APA. This is probably due to enzymic acylation as described by numerous workers with a wide range of micro-organisms[8–11]. The third type of reaction involves the formation of complexes between 6-APA and mould metabolites liberated into the medium. One such metabolite reacting with 6-APA was identified as frequentin, both from a consideration of its properties and by direct comparison with an authentic sample kindly given to me by Dr. W. B. Turner.

Thus when 6-APA was added to a growing culture of an organism producing frequentin a new iodine absorbing[12], penicillinase sensitive zone was detected on chromatograms of the fermentation medium. Chromatography was carried out in butanol/pyridine/water (1:1:1) using Whatman No. 1 paper. Although the new compound was chromatographically similar to the penicillins it had very little activity against *Bacillus subtilis*, and such activity as was observed appeared to increase somewhat variably,

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