TRABAJOS GENERALES

Nucleic acid and single-cell protein utilization in human feeding: A review

JULIO SILVA ARAUJO NETO AND GERSON FERREIRA PINTO
Department of Biochemistry, Institute of Chemistry, Federal University of Rio Janeiro, Brazil.

SUMMARY

Single cell proteins have been treated as a promising way of fighting protein shortage in the world. High nucleic acid content appears as a limiting factor for human utilization. Current studies have intended to find scientific grounds for stating safe intake limits, as well as for obtaining low-purine biomasses. The present review comments the results obtained from nutritional and toxicological assays on which safe intake limits were based and discusses probable reactions of communities toward a SCP supplementation program.

I. INTRODUCTION

Single-cell protein (SCP) has been treated as a promising way of fighting protein shortage in the world. Production of protein from microorganisms does not require large land area and avoids high density agriculture problems (40). A recent critical evaluation on the improvement of world protein nutrition pointed out the advantages of producing and consuming SCP (50). None the less, human utilization of SCP has been limited by psychological, technological and biochemical factors. Among the latter, high nucleic content is a very important one. Kihlberg has recently published two reviews on the use of microbes as a source of food (31). These reviews, as well as books and meetings on SCP have always treated the subject as a whole, without widening the study of high nucleic

---

Recibido: 19-8-1974.

acid intake (31, 49, 53). The SCP nucleic acid content, by itself, is a complex problem and a critical review on several of its aspects could make the introduction of SCP as a major component of some diets easier.

The present paper discusses experiments on human utilization of SCP, particularly related to yeast nucleic acid side effects and intends to subsidize the establishment of safer limits of intake by humans.

II. SCP: CONSUMPTION ALONG THE YEARS AND RESEARCH ON SAFETY

Food yeast, particularly brewer's yeast, has been produced for the past 50 years (4,53), but has been used as a minor component of the diets.

Through the world, some protein-rich mixtures were formulated with yeast: INCAP mixture No. 15 contains 3% (10) and Neston, a Nestle's trade mark in Brazil, is enriched with 1% food-yeast (17). For many years pharmaceutical companies have been producing powders and tablets essentially made with brewer's yeast. Such products, used in dosages from 0.2 to 5 grams/adult.day, are based on yeast vitamin content, but some physicians believe in their therapeutical advantages as related to the treatment of septic dermatosis and gastric upsets. Large amounts of food-yeast were consumed in Germany during the two world-wars (12); scientific grounds, however, are scarce in common bibliography. Some experiments made in Africa have used enriched flours containing heavy proportions of food yeast. Unfortunately, during the experimental program, daily intakes of those mixtures have been too low, neither improving protein diet nor overcharging purine catabolism (3,42). In spite of these poor experiments some experts "expressed their doubt on the advisability of the high yeast content in product" (3). Animal tests are being carried out since 1919 (11). Up to 1960, most of them were focused on the biological value of yeast protein (11,39). The need for better nutritional status in the world led to research on other microbial protein sources. Bacteria (57), fungi (5,35), algae (44) and plankton (6,29) have enlarged this group. Studies related to biomasses harvested from petroleum fractions were soon followed by experiments on toxicological fields. However, up to 1968, only three published papers were essentially designed for toxicological assays (28), all of them working with SCP from petroleum fractions. After 1968, some laboratories have made toxicological tests on common laboratory animals (8,33,48,51). According to the normal evaluation of medicines, food aditives and other non-conventional protein sources, short and long-term toxicity tests were performed. From the whole testing program we conclude that, except for some problems related to absorbed or adsorbed petroleum fractions, biomasses lacked nephro- and liver-toxicity, showed no side-effects on bone-marrow and did not affect the growth of the animals. Nucleic acid content in SCP, however, is much higher than in common food (39) and threats to the human organism arise from it. The ordinary laboratory animals have a different purine metabolism from the human one. Up to 1967, there are not published experiments on higher apes which are simultaneously ureotelic and uric acid excretors by uricase absence (15, 24, 36). Up to now, the very few higher apes tests published have not focused on the problems derived from high purine intakes (13, 48). Pokrowsky (48), in a brief comment, has referred to Russian tests with petroleum SCP; these tests utilized several animal species including monkeys. Indeed, several toxicity aspects were investigated (carcinogenic and leukemogenic effects, fertility rate, side-effects derived from the consumption by animals of biomasses, etc.), but the assessment of purine side-effects was not mentioned. Calloway's paper (13) did not focuse the problems derived from high purine intake: using bacteria as the protein source, she pointed out a bad correlation between safe doses for animals (higher apes or not) and safe doses for men. Several experiments with humans are found in the literature (1, 11, 19, 21, 22, 58). The recent review by Kihlberg on the use of microbes as a source of food (31) quoted some 20 papers looking at the relationship between SCP intake and metabolic disturbances (13, 19): these will be discussed in Section 4.

III. POSSIBLE PROBLEMS DERIVED FROM HUMAN UTILIZATION OF SCP

The mentioned threat about high nucleic acid intake by humans assumes that subjects with a somewhat defective purine catabolism would be affected by overcharging their uric acid excretion system (2, 19, 49). Risks from the general use of high nucleic acid content foods would be (i) as high as the importance of the diet on the onset of dyspurinies, (ii) as important as the number of normal individuals unable to support an overcharging in dietary purines. According to Wallace (60), it has not been accepted that the important clinical consequences, other than urate calculi and gout could be generated by hyperuricemia (60).

3.1 - Onset of dyspurinies v. diet.

Among the syndromes mentioned in the medical literature and truly related to high purine intake, four have to be underlined:

(a) gouty arthritis: primary or secondary, metabolic or renal;
(b) urate calculi: with or without gouty clinical appearance;
(c) gastric or intestinal disturbances;
(d) dermic affections (not proved to derive from antigen-antibody reactions).

These side-effects may be caused by purines, uric acid or by other catabolic compounds.

3.1.1. - Gout v. Dietary purine overcharge.

We cannot get a true and general relationship between purine intake and changes in gout prevalence from the large literature on gout etiopathology. Many years ago gout and genetic defects were correlated (26): it is accepted that gout symptoms are provoked by heterogenous group of genetic abnormalities defined as gout (27). Since presentation of the classical four mechanisms by Gutman and Yu (26) until the most recent papers on the subject, very few published data are useful to speculate on an eventual increase in gout prevalence when the purine intake of the group is more or less increased. Experiments by Gutman and Yu (23), Wingaarden (62), Nugent and Tyler (41), Kaplan (30), Smyth (54) and recent reviews by Sorensen (55) and Samara (52) tell about a large symptomatologic variation which notices very different biochemical facts and discuss various answers to the same dietary or therapeutical scheme. These differences justify the disagreements about the importance of diet participation on gout management. According to Pedrosa (46), "North-Americans are revolted against classical diet, the French —

based on tradition— respect the restricted diet... and Germans use dietary restrictions only in therapeutical failure". Research on large scale on human utilization of SCP have to weigh some increase in the number of hyperuricemic subjects. Gout is indeed recognized as related to an inborn metabolic error and, therefore, quite independent from the environment; however, most of the experts treat dietary purines as an exacerbating factor. In reference to the secondary gout, the origin of which is the enlarged need for purine detoxication, we have to consider that overcharged detoxication may result from myeloproliferation or from the digestion of too much purine foods. It is wise of gouty subjects (assymptomatic or not) and individuals susceptible to secondary gout to be cautious when they are submited to high levels of purine foods during a certain time. On the other hand, it appears logical to believe in a safe ceiling of purine intake: above this level, all people would present too high a serum and urinary uric acid levels.

It appears that a sequential variation of genetic errors would correspond to a sequential diversified clinical appearance, from the typical gouty subject to the common man; when we observe several gouty subjects differing in biochemical factors or in symptoms and other individuals differing in susceptibility to secondary gout, we prefer to say that several existing phenotypes are embraced by the term "gout" (30). Nothing can assure us that any human being may bear every scheme of purine intake. Virtually, after some daily level intake adequately prolonged, all people would develop hyperuricaemia, gout, renal diseases, urate calculi, tophi, etc., without reference to the sequence of onset. Such thinking led to the need of studying and stating safe limits of intake. Virtually, individuals free from any metabolic error would suffer from too heavy a purine intake. Gout statistics, theoretically, would increase step by step in accordance to a stepwise increase in purine intake levels.

3.1.2 - Urate calculi v. Dietary purine overcharge.

A recent study (370 adults) on the serum uric acid (SUA) concentrations in Southern Africa demonstrated that the average SUA levels were similar to those reported for European populations; although a few individuals had SUA levels in the hyperuricemic range, no clinical gout was encountered (7a). These and similar data do not support a close relationship between diet and SUA or between urinary uric acid levels and urate calculi formation. Nevertheless, other facts shows that urinary uric acid levels are essential to urolithiasis. It is true that gouty subjects contribute in 21% to all urate calculi cases (9); on the other hand, 80% of hyperuricemic subjets have articular symptoms (31). A non-direct correlation, however, arises from the question associated with an incorrect organic matrix in urine. In general, only a defective urinary organic matrix allows urate calculi deposition. If a correct organic matrix is present, very large quantities of uric acid would be excreted as "sand" (9,32).

Facts discussed in item 3.1.1. are valid again: the higher the purine intake the more individuals will be able to develop urate calculi and above some level all humans will show the symptom.

3.1.3 - Gastric and dermic disturbances v. High SCP intake.

In early 1959, Goyco's experiments (22) had already reported gastric-intestinal problems in young adults consuming "torula" supplemented diets. The assays studied the supplementary effect of SCP proteins on a Puerto Rican rural diet. Supplementation level was around 15g SCP/adult.day. In spite of the short experimental period (8 days), side-effects were observed: "all stool samples were of a semi-solid consistency and several times stomach discomfort was reported". At the level of 30 g/ adult.day, three patients showed positive nitrogen balance and another one presented diarrhea and vomiting. Miller (39) stated that the most important toxic responses are related to gastric and intestinal facts: at 250 g/ adult.day level all adults consuming algae during a tolerance test presented stomach discomfort and diarrhea.

Abrahamson et coll. (1) relate experiments with healthy adults which consumed SCP "grown on a chemically pure hydrocarbon fraction". Twenty persons consumed for six weeks 20g SCP/person.day and the following conclusions were drawn:

— a woman suffered nausea in the last test week;
— several subjects showed discreet diarrhea or flatulence;
— dermatological problems, high reagin activities (as measured by IgE levels), arthralgies (except a probably traumatic one) were not noted;

— biochemical investigations showed a significant but transient increase in serum uric acid level during the first two weeks.

About dermic problems, Udo et coll. (58) noted that by giving 45, 90 or 135 g SCP per adult daily, during 6 to 11 weeks, 12 subjects "developed moderate, painless peeling of the palms and soles after the first 3 to 4 weeks", although no gastric side-effects arose. Authors pointed out that immunological experiments failed to prove an antigen-antibody reaction. Arroyave's comment (7) on those tests states that dermatological problems disappeared in spite of the maintenance of the dietary scheme (7).

Published data suggest that gastric and intestinal problems are inconsequential for the subjects' future and can always be controlled by interruption break of the dietary supplementation. On the other hand, dermal side-effects appear as a non-solved problem and require more studies. Gastric, intestinal, dermatological and other side-effects from SCP supplementation are frequently related to a careless purification of the harvested cells. According to Edozien (19), "the cause of the wide diversity of views is not clear but variations in the nature of the yeast fed and the substrate on which they were grown are probably the principal factors".

3.2 - Gout prevalence v. Social risks derived from protein/purine supplementation programs.

Hazards from high purine food intake have to be observed in linkage to the epidemiology of dyspurinies. Better statistical data on gout and related diseases prevalence have been claimed, first of all in countries where malnutrition is high (2,56). Gout prevalence varies from country to country and frequently its epidemiological data are presented as a proportion of rheumatological cases surveyed (45, 56, 61, 62). Talbott commented on the poor statistical data available and pointed out that "gouty arthritis is not a rare malady; its recognition, however, leaves much to be desired" (56).

A recent paper by Gorska and Koscianska (63) shows the frequent failure of gout diagnosis: in 25 out of 34 re-examined patients the disease had been treated in a way conductive to further deterioration of the patient's condition.

Primary gout is predominantly a disease of the adult male (9, 54, 61, 62) and is very rare in prepubertal children (54, 62). It has been considered a high —and medium— income groups disease (9, 62). Gout prevalence would be 0.3% in Europe and in the U.S. it has been estimated to be about 275 per 100.000 (62).

More recently, in a New England town, a very well designed survey by O'Sullivan (43) found 3.7 cases per thousand with a 7:1 male to female ratio. Gout prevalence appears to be maximum among some groups in New Zealand, where the greatest protein consumers live. A pilot survey of adults ranked 8.2% of men and 1.6% of women (62).

Different prevalences between the African population (low), and the American negroes (not low), according to Wyngaarden, may include dietary and or ecological influences (62).

Although the developing countries have no adequate surveys, some Brazilian data exist:

(a) Bonomo & Vianna quoted that only 20% of gouty subjects could be classified in low-income groups and that around 0.18% of Brazilians had gout; it was emphasized that 2.5% of the cases were secondary gout and that the figures should increase with improved diagnosis methods (56).

(b) Wettreich listed some data expressing gouty cases as a fraction of rheumatic diseases and showed figures varying from 0.8% to 3.8% (61).

IV. UTILIZATION AND SAFETY: CURRENT STUDIES

Some important ways of getting truly useful biomasses have been proposed. About 1965, preliminary observations opened the possibility of decreasing nucleic acid content of SCP. Less nucleic acid was obtained by using low specific growth rates (20) and growth temperature above the optimum (37). More important results, however, have been achieved by after-growth treatments (14, 16, 18a, 25, 33, 43, 59). Claimed reductions varied from 62% (59) to 90% (14). Should low cost processes be attained in large-scale production, these studies will be of the utmost importance to larger and safer SCP utilization. If 2-3% nucleic acid contents were obtained (dry basis) we would be close to some uncommon but conventional foods (39, 59).

However, until we obtain very low purine biomasses (nucleic acid content similar to the common daily consumed foods) by low-cost processes, the need for safe intake limits will stand up.

After the 39-45 war, research groups began to discuss limits for the consumption of food-yeast: the Medical Research Council Report, (England, 1945) and the German law, which fixed 5% of the daily diet as the maximum intake limit are examplen (12). Fencl, quoting the German data, limited SCP intake to 20g per capita daily, but no further discussion was presented (20). Bunker stated that "doses larger than 15 g or 1/2 ounce can be tolerated and that where ill-effects are manifested it is usually where the material has been newly introduced into the diet and a certain amount of gastric up-st is experienced by some people" (12).

Calloway et coll. fed pure yeast-RNA with purine-free formula diets to healty young men (13). RNA added v. plasma uric acid presented perfectly linear data: to 0, 2, 4 and 8 grams of RNA/day, added plasma uric acid levels were 5.3 ± 0.8, 6.5 ± 0.9, 8.4 ± 1.0 and 10.8 ± 1.3 mg/100 ml. Authors believed that "50% of men can tolerate as much as 2 grams of NA in the daily diet without developing undesirable plasma levels" (13). It is accepted that 7.0 mg/100 ml is the upper limit dividing non-gouty from gouty subjects.

Udo et coll. gave up to 135g of dried food grade yeast to universitary studients during up to 12 weeks (13): less than 10% of the subjets submitted to a 45g yeast/day diet reached 7.0g of uric acid/100ml of plasma (19). Therefore, the acceptable maximum amount, which could be safely tolerated is less than 45g.

Edozien (19) believes that the safe limit is probably around 2g yeast-RNA per adult daily. Based on Calloway's and Edozien's data (13, 19), the Protein Advisory Group of the United Nations System (PAG/UN) have stated that "the currently available information suggests that there should be a limit of two grams per day on the amount of nucleic acid introduced by SCP into the diet of an adult" (49).

More recently, Pinto & Araujo Neto presented a different approach to state SCP intake limits (47): normal purine intakes (an non supplemented diet) from high and low-income groups were found different and the difference was taken as a basis for this statement. Pinto & Araujo Neto's "Natural limit of safety" has been stated only for a focused and nutritionally surveyed area and allows an extra purine level (given to low income group) which would put previous low-income group purine intake equal to normal high-income-group purine intake. This approach calculated for two Brazilian rural areas puts SCP intake limits between 10-20 grams percapita daily (47). PAG/UN limits for children is the adult limit reduced on a body weight basis (49). Pinto & Araujo Neto believed that their "natural limit safety", after carrying out children nutritional survey, could be safer and more meaningful.

We must emphasize that a recent report of the PAG "ad hoc" working group on SCP demonstrates that SCP nucleic acids should not be regarded as a nutritional threat. This report only points out need for computing the nucleic nitrogen while evaluating protein value of SCP (3a).

V. FINAL REMARKS

Biochemical and toxicological problems are commonly found in conventional food sources (18, 34, 39). Some of them are overcome by processing, other by fitting the organisms by limiting intake levels or by careful raw material selection. Observed side-effects in SCP experiments in humans often related to the substrates which were used or to the failure of the final phase of processing. Published studies on SCP toxicity are limited but suggest controlled utilization. Probably any human group which increases its purine untake will also increase its purine-related diseases prevalences. Such an increase can be explosive or can be a controlled one. By using SCP the controlled increase can contribute to improvement the nutritional status and can reduce uncommon or unacceptable purine-related diseases. The classical relationship between "dyspurinies" (particularly gout) and high-income-well-nourished-groups says that an increase in purine-diseases could appear whenever under-nourished groups have their nutrition improved by any means. Unwise dread must give place to a balance between the warranted benefits from better protein feeding and the risks from increased purine-diseases prevalences. Research on low-nucleic acid biomasses may succeed in a few years. Studies on safe intake limits allow already SCP supplementation schemes which do contribute to improve protein nutrition in many communities. It is noteworthy that studies on SCP safe limits are all concordant and we can consider 10-15 grams SCP/adult. day as a very safe doses (12, 13, 18, 20, 47, 49). However, problems related to intake limits for children have been scarcely considered.

RESUMEN

Revisión sobre la utilización de proteínas unicelulares en alimentación humana.

Los organismos unicelulares han sido considerados como un recurso promisor para combatir la escasez mundial de proteínas. Su alto contenido en ácidos nucleicos constituye un factor limitante para su empleo en alimentación humana. Las investigaciones sobre este particular han tratado de establecer bases científicas para definir cantidades inocuas de ingesta así como también para obtener biomasas de bajo contenido en purinas. En la presente revisión se comentan los resultados obtenidos en ensayos nutricionales y toxicológicos que han dado base para sentar los límites de ingesta y se discuten probables reacciones de grupos de población a un programa de suplementación con proteínas unicelulares.

BIBLIOGRAPHY

1. Abrahamson, L., L. Hambraeus, Y. Hofvander and B. Vahlquist. Single cell protein in clinical testing. **Nutr. Metabol.**, 13: 186, 1971.
2. Anonymous. Safety of single cell protein, **PAG Bulletin**, 1: 3, 1970.
3. Anonymous. Protein food mixtures in Africa. **PAG Bulletin**, 1: 32, 1969.

3a. Anonymous. Report on the third meeting of the PAG "ad hoc" working group on single-cell protein, **PAG Bulletin**, 3: 1, 1973.

4. Anonymous. Resolution on single-cell protein. **PAG Bulletin**, 1: 15, 1969.
5. Anonymous. Single-cell protein-research and development. **PAG Bulletin**, 1: 8, 1971.
6. Anonymous. Valeur alimentaire du placton marin. **La Presse Med.**, 71: 2179, 1963.
7. Arroyave, G. The discussion in **Protein-Calorie Malnutrition**, Berlin, Springer-Verlag, 1969. Pag. 5.

7a. Beighton, P., L. Salomon, C. L. Soskolne and B. Sweet. Serum and concentrations in a rural Tswana Community in Southern Africa. **Ann. Rheum. Dis.**, 32: 346, 1973.

8. Bennet, I. C., J. R. Todd and J. C. Hondermarck. How B. P. makes proteins from hydrocarbons. **Hydroc. Proc.**, 48: 108, 1969.
9. Bevacqua, V. and A. M. Somajni. The gout - part II. **Res. Clin. Cient.** (Sao Paulo), 39: 109, 1970.
10. Bressani, R. Formulated vegetable mixtures in: **Proceedings of Western Hemisphere Nutrition** Congress-I, Chicago, American Med. Assoc. 1966, Pag. 89.
11. Bressani, R. **The use of Yeast in human foods, in Single cell protein**, Cambridge, MIT Press, 1968, Pag. 90.

12. Bunker, H. J. **Sources of Single cell protein: perspective and prospect in Single cell protein**, Cambridge, MIT Press, 1968, Pag. 67.
13. Calloway, D. H. Safety of single cell protein. Document 2.32/2, New York, Protein Advisory Group of the United Nations System, 1969.
14. Canepa, A., M. Pieber, C. Romero and J. C. Toha. A method for large reduction of the nucleic acid content of yeast. Biotechn. Bioengn., 14: 173, 1972.
15. Cantarow, A. and B. Schepartz. **Biochemistry.** Philadelphia, W. B. Saunders Co., 1967.
16. Castro, A. C., A. J. Sinskey and S. R. Tannenbaum. Reduction of nucleic acid content in Candida yeast cells by bovine pancreatic ribonuclease A treatment. **Appl. Microbiol,** 22: 422, 1971.
17. Cía. Industrial e Comercial Brasileira de Produtos Alimentares (Nestlé Co., Brazil). Pediatric data and Nestlé vademecum. Sao Paulo, 1970.
18. Coon, J. M. Naturaly occuring toxic materials in: **Proceedings of Western Hemisphere Nutrition Congress-I**, Chicago, Amer. Med. Association, 1960, Pag. 136.
18a. Daly, W. H. and L. P. Ruiz. Reduction of RNA in SCP in conjunction with fiber formation. **Biotechn. Bioengn.** 16: 285, 1974.
19. Edozien, J. C. Yeast for human feeding - new data on safety. Document 2.23/1, Geneva, Protein Advisory Group of the United Nations System, 1969.
20. Fencl, Z. **Production of microbial protein from carbon sources in Global Impacts on Microbiology-II**, New York, Interscience Publ., 1969, Pag. 63.
21. Floch, H. Résultats d'essais d'utilisation de lévures alimentaires en Guyane Française. **Nutr. Abst. Rev.**, 26: 471, 1956.
22. Goyco, J. A. Nitrogen balance of young adults consuming a deficient diet supplemented with torula yeast and other nitrogenous products, **J. Nutr.**, 69: 49, 1959.
23. Gutman, A. B. and T. F. Yu. Prevention and treatment of chronic gouty arthritis. **J. A. M. A.**, 157: 1096, 1955.
24. Harrow, B. and A. Mazur. **Textbook of biochemistry.** Philadelphia, W. B. Saunders Co., 1966.
25. Hedenskog, G. and L. Ebbinghans. Reduction of the nucleic acid content of SCP concentrates. **Biotechnol. Bioengn.**, 14: 447, 1972.
26. Hsia, D. Y., M. O'Flynn and H. White. Hereditary metabolic diseases of bones and joints in: **The Medical Clinics of North America**, v. 49, Philadelphia, W. B. Saunders Co., 1965, Pag. 281.
27. Hsia, D. Y. Study of hereditary metabolic diseases using **in vitro** techniques. **Metab. (Baltimore)**, 19: 326, 1970.
28. Humphrey, A. E. Production of protein from petroleum in: **Proceedings of the Western Hemisphere Nutrition Congress-II**, Chicago, American Medical Association, 1969, Pag. 91.
29. Jaffé, W. Contribución al estudio del valor nutritivo del plankton. **Arch. Venez. Nutr.**, 9: 147, 1959.

30. Kaplan, G. Aspect clinique et biologique des dyspurinies avec deficit enzymatique. **Path. Biol.**, 18: 881, 1970.
31. Kihlberg, R. The microbes as a source of food. **Ann. Rev. Microb., 26:** 427, 1972.
32. Klinenberg, J. R., R. Bluestone, L. Schlosstein, J. Waisman and M. W. Whitehouse. Urate Deposition Disease: how it is regulated and how can it be modified? **Ann. Int. Med.** 78: 99, 1973.
33. Ko, P. C. and Y. Yu. Production of SCP from hydrocarbons in: **Single cell protein,** Cambridge, MIT Press, 1968, Pag. 255.
34. Leopold, A. C. and R. Ardrey. Toxic substances in plants and the habits of the early man. **Science,** 176: 512, 1972.
35. Litchfield, J. H. The production of fungi in: **Single cell protein,** Cambridge, MIT Press, 1968, Pag. 309.
36. Mahler, H. R. and E. H. Cordes. **Basic biological chemistry,** Tokyo, Harper & Row - John Weatherhill Inc.., 1968.
37. Mateles, R. I. Applications of continuous culture in: **Single cell protein,** Cambridge, MIT PRESS, 1968, Pag. 208.
38. Maul, S. B., A. J. Sinskey and S. R. Tannembaum. New process for reducing the nucleic acid content of yeast. **Nature,** 228: 181, 1970.
39. Miller, S. A. Nutritional factors in single cell protein in: **Single cell proteins,** Cambridge, MIT Press, 1968, Pag. 79.
40. Mark, E. M. Food technology in: **Proceedings of the Western Hemisphere Nutrition Congress-I,** Chicago, American Medical Association, 1969, Pag. 229.
41. Nugent, C. C. and F. H. Tyler. The renal excretion of uric acid in patients with gout and in non-gouty subjects. **J. Clin. Invest.,** 38: 1890, 1959.
42. Odendaal, W. A. Pronutro. **PAG Bulletin,** 1: 13, 1965.
43. Ohta, S., S. Maul, A. J. Sinskey and S. R. Tannembaum. Characterization of a heat shock process for reduction of the nucleic acid content of **Candida utilis. Appl. Microb.,** 22: 415, 1971.
44. Oswald, W. J. and C. G. Golueke. Large-scale production of algae in: **Single cell protein,** Cambridge, MIT PRESS, 1968, Pag. 271.
45. O'Sullivan, J. B. Gout in a new England town. **Ann. Rheum. Dis., 31:** 166, 1972.
46. Pedrosa, X. Comment (Symposium on gout and metabolic arthropaties). **Brasil Med. (Rio de Janeiro),** 73: 344, 1959.
47. Pinto, G. F. and J. S. Araujo Neto. Limits for single cell protein utilization in human feeding. **Arch. Latinoamer. Nutr.,** 22: 49, 1972.
48. Pokrowsky, A. A. Toxicological studies on single cell protein (a comment) in: **Single cell protein,** Cambridge, MIT Press, 1968, Pag. 163.
49. Protein Advisory Group of the United Nations System. PAG Statement on single cell protein. **PAG Statement Nº 4, New York,** 1970.
50. Protein Advisory Group of the United System. PAG Statement on the world protein problem. **PAG Statement Nº** 12, New York, 1971.
51. Rey, L. and J. Mauron. Single cell protein: basic aspects and future trends in: **Protein-Calorie Malnutrition,** Berlin, Springer-Verlag, 1969, Pag. 86.

52. Samara, A. M. Aspectos clínicos e terapeuticos da gota. **Clin. Geral.** (Sao Paulo), 6: 36, 1972.
53. Scrimshaw, N. Introduction. α in: **Single cell protein**, Cambridge, MIT Press, 1968, Pag. 3.
54. Smyth, C. J. Hereditary factors in gout. **Metab. (Baltimore)**, 6: 221, 1957.
55. Sorensen, L. B. Current concepts of gout and its treatment in: **The Medical Clinics of North America**, v. 47, Chicago, W. B. Saunders Co. 1963, Pag. 169.
56. Talbott, J. H. **Gout and gouty arthritis.** New York, Grune & Stratton, 1953.
57. Tannembaum, S. R., R. I. Matels and G. R. Capco. Processing of bacteria for production of protein concentrates in: **Advances in Chemistry**, v. 57, Chicago, American Chemical Society, 1966, Pag. 254.
58. Udo, U., V. Young, J. Edozien and N. Scrimshaw. Evaluation of torula yeast for human consumption. **Fed. Proc.**, 28: 807, 1969.
59. Villalon, R. and M. A. Tagle. Eliminación parcial del RNA de levaduras. **Arch. Latinoamer. Nutr.**, 21: 307, 1971.
60. Wallace, S. L. The treatment of gout, **Arth. Rheum.**, 15: 317, 1972.
61. Wettreich, W. Comment (Symposium on gout and metabolic arthropaties). **Brasil Med. (Rio de Janeiro)**, 73: 346, 1959.
62. Wyngaarden, J. B. Etiology and pathogenesis of gout in: **Arthritis and allied conditions**, Philadelphia, Lea & Febiger Co. 1967, Pag. 903.
63. Zimmerman-Gorska, I. and J. Koscianska. Problems of diagnostic failure in gout arthritis. **Ann. Clin. Res.**, 4: 95, 1972.