for the five mouse tumor types reported on averaged only −7.1! Three of these types (sarcoma 37S, tar carcinoma 2146, and spindle-cell tar tumor 173) had an average Q_{O_2} of −5.6, compared with −16.6 for the *same* tumor strains as measured by Crabtree and used by Weinhouse. Thus, even the *unadjusted* average tumor Q_{O_2} value employed by Weinhouse (−11.8) is, as the result of unwarranted bias in selection and incomplete utilization of available data, much too high.

11. Weinhouse's confusion is infectious, although mainly among investigators who do not themselves perform laboratory experiments on cancer metabolism. One may read, for example, "We hear about THE metabolism of THE cancer cell. Unfortunately, no such phenomenon has been established. . . . As Dr. Weinhouse said at the opening of the symposium, the critical difference between metabolism in malignant tissues and in normal tissues does not appear to reside in the major ways in which they handle carbohydrate metabolism." [*Antimetabolites and Cancer* (AAAS, Washington, D.C., 1955, pp. 305, 308)]. Such statements could scarcely be more incorrect or uninformed. They set the clock back and encourage the empirical approach to the problem of cancer by a sheer and vicarious denial of available fundamental information.
12. G. N. Lewis, *The Anatomy of Science* (Yale Univ. Press, New Haven, Conn., 1926), p. 171.
13. In paragraphs 4, 5, and 6 of his note in this issue of *Science*, Weinhouse asks or raises several questions that have been asked, discussed, and answered many times in the literature of cancer.
14. The mechanism of the cancer respiratory impairment may indeed often involve lowered content of a particular respiratory enzyme, but this is not a necessary general requirement, since internal cellular arrangement and chemical or structural restraint of other correlated enzymes are, as in so many living phenomena, often of more decisive importance. Thus, certain ascites cancer cells have been found [B. Chance and L. N. Castor, *Science* 116, 200 (1952)] to have unusually high contents of cytochrome *c*; but even in such ascites cells, the paraphenylenediamine and succinate oxidative responses are characteristically low or zero (*1*, p. 314); this is clearly indicative of respiratory restraint in spite of abnormally high absoute content of cytochrome *c* (compare *4*, pp. 295–6). Oxidation-reduction potential restraints may well be involved here, as well as low contents of cytochrome *b* or DPNH demonstrated.
15. I. Newton, *Opticks* (W. and J. Innys, London, ed. 2, 1718), pp. 344, 351.
16. Presented orally at the 1956 meeting of American Association for Cancer Research. [*Proc.* 2, 98 (Natl. Inst. of Health Information Release, 13 Apr.)].
17. A description of these quantitative potentialities and other qualitative aspects is in preparation.

2 July 1956

On the Biosynthesis of the Porphyrinlike Moiety of Vitamin B_{12}

The investigations performed during the past decade have elucidated many of the intimate biosynthetic steps by which the cell elaborates the porphyrin molecule. It has been found that "active" succinate (*1*) and glycine (*2*) are the sole precursors of the porphyrin compounds in all biological systems studied. The glycine and succinate condense to form α-amino-β-ketoadipic acid. This β-keto acid on decarboxylation yields δ-aminolevulinic acid (*3*). Condensation of 2 mole of the aminoketone results in the formation of the precursor monopyrrole, porphobilinogen (*4*). Four mole of this pyrrole then condenses to form a porphyrin, and modification of the side chains in the β-positions gives rise to a particular porphyrin.

The chemical work of the Merck group (*5*) and of the English workers (*6*) and the x-ray studies of Hodgkin *et al.* (*7*) have culminated recently in the proposal of a very probable structure of vitamin B_{12} which contains a porphyrinlike structure (*6, 7*). Although this latter component of the vitamin differs somewhat in structure from that of porphyrins (that is, the vitamin molecule contains one pyrrolidine and three pyrroline rings, a methyl group on two of the bridge-carbon atoms, four extra methyl groups in the β-positions of the rings, and an α-methyl group instead of a bridge-carbon atom), there are sufficient similarities to lead to the suspicion that the basic mechanism of synthesis of this part of the vitamin is similar to that known for porphyrins. It would seem possible that the porphyrinlike moiety of the vitamin is synthesized by the mechanism known for pyrrole and porphyrin synthesis and that the modified structure is subsequently methylated in the afore-mentioned positions to form the final product. This conclusion, of methylation subsequent to ring formation from δ-aminolevulinic acid (*6*), is supported by structural considerations. If a methylated derivative of δ-aminolevulinic acid were the precursor, one would expect that the extra methyl groups would be on only those β-positions that bear acetic acid side chains. However, this is the case with only rings A and B; in ring D the methyl group is attached to the carbon atom that bears the propionic acid group.

In order to check this hypothesis, we have carried out a microbiological synthesis of vitamin B_{12} in the presence of 125 mg of δ-aminolevulinic acid-1,4-C^{14} having a molar activity (*1*) of 8.3×10^5 count/min for each active carbon. The culture was agitated in a medium containing the following nutrients, in addition to the δ-aminolevulinic acid: sucrose, 8.75 g; L-glutamic acid, 2.5 g; $(NH_4)_2HPO_4$, 0.5 g; Na_2SO_4, 0.5 g; KCl, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.125 g; $MnSO_4 \cdot 4H_2O$, 0.05 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; $ZnSO_4 \cdot 7H_2O$, 0.005 g; and $Co(NO_3)_2 \cdot 6H_2O$, 0.01 g. Under these fermentation conditions, the culture produced 0.163 mg of vitamin B_{12}. After the addition of 10.1 mg of nonradioactive B_{12}, 6.294 mg of B_{12} was isolated. The molar activity of the undiluted B_{12} was 30×10^5 count/min. Therefore, in the unlikely possibility that endogenous synthesis of aminoketone be disregarded, at least four carbon atoms of the vitamin must have contained C^{14}.

On the reasonable assumption, based on previous studies on porphyrin formation, that 2 mole of aminoketone is utilized for each ring, one can postulate that 15 labeled carbon atoms (16 minus the carboxyl lost from ring C) of the porphyrinlike structure of the vitamin were derived from our labeled substrate. On this basis the molar activity of each of these 15 carbon atoms would be 2×10^5 count/min. This represents a mere fourfold dilution of the radioactive carbon atoms of the labeled substrate in the course of the synthesis of the vitamin. It may therefore justifiably be concluded that the porphyrinlike structure of vitamin B_{12} is synthesized from δ-aminolevulinic acid, as are the porphyrins, and that the mechanism of synthesis of the ring system in the vitamin is similar to that of the porphyrins.

We are presently engaged in degrading the labeled vitamin in order to isolate those carbon atoms which we predict should contain all the radioactivity.

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28 May 1956

Cell-Wall Mannan-Protein of Baker's Yeast

In isolated cell walls of baker's yeast, Northcote and Horne (*1*) demonstrated the presence of two polysaccharides, an outer glucan envelope and an inner mannan component. Associated with the latter was nitrogenous material. This material was assumed to be protein on the basis of the detection (by chromatography) of amino acids in the products of its partial hydrolysis. The glucan was subsequently shown (*2*) to comprise about 10 percent, and mannan 15 to 17 percent, of the dry weight of baker's yeast. The wall constituents accounted

for approximately 30 percent of the dry weight of the cells.

By mechanical disruption of baker's yeast and its differential centrifugation in media of differing density, preparations of isolated cell walls have been obtained that are entirely free from intact cells and from other particulate cellular matter. From the clean cell-wall fragments, we have isolated a mannan-protein complex (*3*) that appears to be a major structural entity of the cell.

Cells from starch-free pound cakes of baker's yeast (*4*) suspended in water or in a 5 percent (by volume) aqueous solution of thiodiglycol (150 g of yeast, 50 ml of solution) were disrupted in the cold by agitation in a Waring Blendor with glass beads according to the method of Lamanna and Mallette (*5*). More than 90-percent breakage of cells was achieved after 90 minutes of agitation. Cell-wall fragments were isolated by differential centrifugation and repeated washing with distilled water, 8.5-percent sucrose solution, and phosphate buffer (*p*H 7.4, 0.1*M*). Washing with sucrose and with buffer served to eliminate completely the small particles which, in water, sediment along with cell-wall fragments.

After about 50 repetitions of washing and differential centrifugation, a fraction consisting exclusively of cell-wall material was obtained. This was lyophilized. All data are based on material prepared in this manner. By this method of preparation, it is possible to obtain a cell-wall fraction of constant composition. From two different batches of yeast, the nitrogen contents of the cell-wall preparations were 1.28 and 1.24 percent. These values are lower than that (2.1 percent) reported by Northcote and Horne, who stated that their preparation contained no unbroken cells but was contaminated by small particles which were difficult to eliminate.

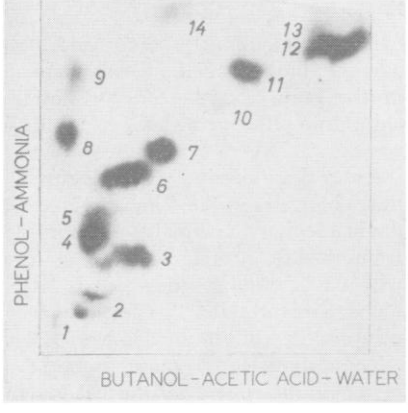

Fig. 1. Amino acids in hydrolyzates (6*N* HCl for 16 hours at 110°C) of isolated cell wall of baker's yeast as demonstrated by two-dimensional paper chromatography (solvents were a solution of butanol, acetic acid, and water and a solution of phenol and ammonia; ninhydrin development; cobalt chloride spray to intensify color and make record permanent): *1*, cysteic acid; *2*, aspartic acid; *3*, glutamic acid; *4*, serine; *5*, glycine; *6*, threonine; *7*, alanine; *8*, lysine; *9*, arginine; *10*, tyrosine; *11*, valine and methionine; *12*, leucine and isoleucine; *13*, phenylalanine; *14*, unidentified. Small amounts of hexosamine present were not detectable after vigorous hydrolysis.

Table 1. Composition of isolated cell wall of baker's yeast.

Component	Percentage by weight of dry cell wall
Total reducing sugar	84.4
Hexosamine	2.7
Total nitrogen	1.28
Hexosamine N	0.21
Protein N	1.07
Protein N (×6.25)	6.7
Phosphorus	0.34
Sulfur	0.14

The analytic data presented in Table 1 show that the clean cell-wall material contains 6.7 percent protein, 84.4 percent total reducing sugar (anthrone method, *2*), and 3.0 percent chitin (based on the hexosamine content). The high sulfur-to-protein ratio (2.1 percent) indicates that the protein may be of a pseudokeratin type (*6*). By means of paper chromatography of hydrolyzates of cell-wall material, the presence of 15 amino acids and one as yet unidentified substance was demonstrated (Fig. 1).

From preparations of clean cell-wall materials a major fraction (approximately 75 percent) was solubilized in 1*N* KOH on slight warming. The solubilized material was dialyzed against running tap water for 24 hours and then lyophilized, yielding a white powder, of which the major part was readily soluble in water. The residue formed a gel that was separated from the solution by centrifugation at 20,000*g*. The nature of this fraction is under study; it appears to be a glucan-protein. (Only glucose is found, in addition to amino acids, after acid hydrolysis). The water-soluble material that had resisted dialysis was found to contain mannan and protein, as shown by analysis for tyrosine (Folin) and polysaccharide (anthrone), ultraviolet absorption at 280 mμ and chromatographic analysis (Only mannose is found, in addition to amino acids, after acid hydrolysis.) Nucleic acid was absent as judged by absorption at 260 mμ. Three different preparations had nitrogen contents close to 1.10 percent (equivalent to 6.8 percent protein).

The mannan and protein appeared to be tightly bound, for the polysaccharide could not be precipitated as the copper complex by treatment with cold Fehling's reagent (*7*). In the ultracentrifuge, the mannan-protein appears to be monodisperse, giving at this stage of purification a sedimentation constant (uncorrected) of $S_{20} = 4.3 \times 10^{-13}$ at several concentrations in water and in buffer (*8*). The mannan-protein complex has been further purified by precipitation from aqueous solution on saturation with ammonium sulfate followed by dialysis and subsequent lyophilization. The amino acid composition of this material is being analyzed quantitatively, and biochemical, immunochemical, and physical studies on the mannan-protein are underway.

From fresh bottom yeast (brewer's yeast) Lindquist (*9*) obtained, by simple agitation of a thick suspension at room temperature, a material which he termed "cebrosan" that has some features in common with the mannan-protein we have isolated. Lindquist reported his purified material contained 1.2 percent nitrogen, but it had the property of a yeast mannan—that is, it was precipitable by Fehling's solution in the cold. The material we have isolated appears to be an almost homogeneous structural entity of the cell wall, consisting of protein and mannan in the ratio (by weight) of 1/12. Evidence has been obtained that this cell-wall protein plays a role in cellular division (*10*).

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Inhibitory Mechanism of Chlortetracycline on D-Amino Acid Oxidase

Yagi *et al.* (*1*) found that *p*-aminosalicylic acid quenched the fluorescence of riboflavin in aqueous solution by the formation of a complex and that the inhibitory action of *p*-aminosalicylic acid on D-amino acid oxidase is partly due to the complex formation between