TABLE I

AGGLUTINATION TESTS WITH SERUM OF RABBITS IMMUNIZED WITH COLLODION PARTICLES ADSORBED WITH ANTHRAX POLYSACCHARIDE AND OTHER COMBINATIONS

Injected with		C. particles and anthrax SSS			C. particles and antianthrax Gl. and anthrax SSS			C. particles and normal horse serum and anthrax SSS			Anthrax Gl.-anthrax SSS ppte		
	Rabbit No.	6704	6705	6706	6707	6708	6709	6713	6714	6715	6710	6711	6712
Final serum dilution	1/4	4	4	3	4	4	3	4	4	4	4	4	4
	1/8	4	3	2	4	4	3	2	3	4	4	4	4
	1/16	4	2	2	4	4	2	1	3	2	4	2	4
	1/32	4	1	1	2	4	1	0	2	1	1	0	3
	1/64	2	0	0	2	2	0	0	0	0	0	0	1

Incubated over night in water bath at 37° C.

sorbed on the particles is available for stimulating antibody response.

These results may offer an explanation of why polysaccharides and other substances (of the class of haptenes) are not antigenic when injected alone in solution in the body. The collodion particles provide an enormously increased surface to the polysaccharide (haptene) and make them available in a different physical form on injection. Other factors no doubt come in.

Further work is being done on many of the problems here suggested, especially in finding other colloids which may be employed for the same purpose, as well as the immunization of different animals with various bacterial polysaccharides. Work is in progress with the protection of mice against pneumococci with the specific carbohydrate to collodion particles. At the same time we have adsorbed several polysaccharides in the same collodion particles with the hope of forming a multiple antigen. The result of all this work will be published later.

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LIGNIN AS A NUTRIENT FOR THE CULTIVATED MUSHROOM, AGARICUS CAMPESTRIS

THE cultivated mushroom, *Agaricus campestris,* in common with other fungi, must obtain all the nutrients necessary for its growth and reproduction from the organic matter of the substrata upon which it grows, this organic matter also serving as a source of energy. Horse manure is commonly used as the source of organic matter for the cultivation of the mushroom, although recently attempts have been made to replace this manure by composts of so-called "artificial manure" prepared from straw and other plant residues, from peat, etc. The chief point to be considered in the use of manure or composts of plant residues is that, in order to obtain a fair growth of the mushroom, the manure and residues must first be thoroughly composted; this process lasts, under favorable conditions, from 4 to 12 weeks. The microorganisms active in the composts bring about a number of chemical changes in the various organic complexes; these can be briefly summarized as a reduction of the water-soluble substances, of the hemicelluloses and cellulose, and an increase in the lignin, ash and protein content.

The problem is to determine which of these chemical constituents in the compost form the nutrients for the mushroom, whether all the organic complexes are attacked alike, or whether some are acted upon to the exclusion of others. This problem is both of theoretical interest and has considerable practical application.

It is known, from studies of the activities of wood-destroying fungi, that some organisms attack the cellulose of the wood, but not the lignin, while a few are capable of attacking the lignin as well as the cellulose. With the exception of these wood-destroying fungi, very few organisms are known so far capable of decomposing lignin and using this material as a source of energy and as a nutrient material. Although lignin has been shown to be generally much more resistant to decomposition by microorganisms than celluloses and hemicelluloses, as shown by its relative persistence in composts, peat bogs and soil, it still undergoes some decomposition, especially under aerobic conditions; otherwise the whole surface of the earth would soon be covered with lignin, since all plant residues, with the possible exception of the algae, contain from 5 to 30 per cent. lignin.

In order to illustrate the changes that take place in the composting of stable manure, a typical analysis of a compost of horse manure is given Table I. If one assumes that the concentration of the mineral

TABLE I

CHANGES IN THE CHEMICAL COMPOSITION OF HORSE MANURE PRODUCED BY MICROORGANISMS IN THE PROCESS OF COMPOSTING

Chemical constituent	Fresh manure	Compost 27 days old	Compost 108 days old
Total nitrogen	1.58	2.05	2.62
Ether soluble portion	1.85	1.28	0.70
Cold water soluble portion	4.45	6.64	5.11
Hot water soluble portion	2.95	7.43	3.85
Hemicelluloses	18.84	15.26	11.74
Cellulose	27.41	20.41	12.00
Lignin	17.69	19.98	22.30
Water-insoluble protein	8.25	11.50	13.56
Ash	12.51	17.80	25.50

constituents of the manure increase in inverse proportion to the reduction of the organic constituents, the doubling of the ash content of the compost within 108 days indicates a reduction of the total organic matter to one half of the original material. Of the four most important groups of chemical complexes of the manure, namely, the hemicelluloses, cellulose, lignin and protein, the first two diminished in relative concentration, especially the cellulose, while the last two increased in proportion. It is these two complexes, namely the lignin and the protein, which are used predominantly by the mushroom fungus for their growth and activities, as shown in Table II.

TABLE II

DECOMPOSITION OF THE VARIOUS CHEMICAL CONSTITUENTS OF FRESH AND COMPOSTED MANURE BY AGARICUS CAMPESTRIS

Chemical constituent	Fresh manure		Composted manure			
	Proximate composition per cent. of dry material		Total amount in culture per cent. of original	Proximate composition per cent. of residual dry material		Total amount in culture per cent. of original
	Control	Culture		Control	Culture	
Total material	.....	.....	94.0	.....	.....	90.6
Total nitrogen	1.44	1.65	107.2	1.5	1.6	96.0
NH_3–N per cent. of total N	.....	.....	.....	2.7	5.2	192.6
Ether-soluble	2.3	1.4	57.2	0.7	0.6	72.9
Hot water soluble organic matter	5.6	11.9	199.3	3.6	10.5	264.7
Water soluble nitrogen per cent. of total nitrogen	25.3	40.0	.....	15.2	39.4	.....
Hemicelluloses	18.6	14.4	72.8	7.1	6.6	83.2
Cellulose	20.7	20.8	94.5	14.8	16.7	101.9
Lignin	20.0	15.7	73.8	20.8	14.8	64.5

It is important to note that, as a result of the growth of *Agaricus campestris* upon fresh and composted manure (these experiments were carried out by inoculating sterilized manure with pure cultures of the organism), there was very little reduction in the total weight of the compost; the actual loss was only 6 to 9.4 per cent. of the original material. This reduction is only apparent, since the mushroom fungus synthesizes an extensive quantity of mycelium, which compensates for a large part of the organic matter of the compost which has been lost through decomposition. This is illustrated by the marked increase of the water-soluble organic matter and the nitrogen as a result of the growth of the fungus; the increase in these complexes is due entirely to the fact that over 40 per cent. of the mycelium of the fungus, on a dry basis, is water soluble.

Of the more important organic complexes, the cellulose was either not attacked at all by the mushroom, as in the case of the composted manure, or reduced only to a limited extent. The hemicelluloses were markedly reduced, especially in the fresh manure. The most striking phenomenon is observed in the reduction of the lignin, which forms the most abundant group of organic complexes in the composted manure and of which more than one third was decomposed by the mushroom in 47 days. The reduction of the lignin in the composted manure accounts, in these experiments, for nearly 80 per cent. of the total reduction in the weight of the original material. There is no doubt that the protein of the compost has also undergone considerable change, as shown by the increase in ammonia and in water-soluble nitrogen. The fact that the mushroom contains about 6.5 per cent. total nitrogen, a large part of which is soluble in water, is responsible for this.

One may, therefore, feel justified in concluding that *Agaricus campestris* feeds largely upon the lignin and protein of the manure and to a less extent upon the hemicelluloses, cellulose and other complexes. Further, that the need for the composting of manure, in order to develop a favorable medium for the growth of mushrooms, consists in bringing about an enrichment in the lignin and protein content and possibly in a change in their chemical nature.

A detailed discussion of these experiments and their bearing upon the problem of mushroom nutrition will be published later.

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