

and the contents of the blender are being actively mixed. Thirty-three rat carcasses were analyzed for moisture, protein and energy using this technique of sampling. The coefficients of variation (*i.e.*, variation among samples, within animals) found for analytical values of homogenates and derived analytical values for fresh carcasses varied from 0.25 to 1.19%. The largest coefficients of variation were found for per cent protein of homogenates (1.19%) and of fresh carcasses (0.95%). Other coefficients of variation observed were: per cent moisture in homogenates and fresh carcasses, 0.25 and 0.58%; and calories/g of homogenates and fresh carcasses, 0.32 and 0.34%, respectively.

We wish to thank Mr. John A. Weaver for his assistance with protein and energy analyses, and Dr. K. R. Bennett for his suggestions concerning presentation of statistical data.

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Received April 29, 1963. P.S.E.B.M., 1963, v113.

Tracheal Muscle Contracting Action of Metabolites Produced by Air-Borne Fungi. (28549)

S. OHASHI, M. YAMAGUCHI, I. YAMAMOTO AND Y. KOBAYASHI
(Introduced by K. K. Chen)

Department of Pharmacology, Nippon Medical School, Tokyo, Public Health Research Institute of Kobe, and Department of Pharmacology, Faculty of Medicine, University of Tokyo, Japan

Many experiments have been performed dealing with the antibiotic action of the metabolites of air-borne fungi, which greatly affect humans in their daily lives, and with the antigenic action of their crude extracts. In studying the effect of such metabolites on the living body, the authors observed the action of the metabolites on tracheal muscles of guinea pigs, for it was assumed that some special substances contained in these fungus metabolites might be connected with bronchial asthma attacks. In this paper are presented *in vitro* experiments on the action of air-borne fungus metabolites on the tracheal muscles of guinea pigs.

Methods. 1) *Tracheal muscle preparation.* Guinea pigs weighing 300-400 g were killed, the tracheas (from the sublaryngeal part as far as the bifurcation) were removed and tracheal muscle preparations were prepared according to the method of Takagi *et al.*(1).

The preparations were suspended in Magnus-tubes filled with Locke-Ringer's solution maintained at 38°C and continuously sup-

plied with oxygen. Contraction and relaxation of the tracheal muscle were recorded on a kymograph. Each metabolite was added into the medium at various concentrations. During the experiment the medium was maintained at pH 7.2.

2) *Collection and isolation of air-borne fungi.* Dust and air-borne fungi from a room of 4 bronchial asthma patients were cultivated in Sabouraud's medium, then each fungus was cultured in the selected medium for isolation.

Results and discussion. 1) *Isolated fungi.* As shown in Table I, 120 fungus strains were isolated. 2) *Action of the culture broth on tracheal muscle.* Culture broths of all the isolated fungi were tested for their effect on the tracheal muscle preparations. The culture broths were diluted in 10 volumes of Locke-Ringer's solution when used. The culture broths were evaluated as to whether they involved contraction, relaxation, or no effect (Table II). Since the culture broth may contain various substances, no categori-

TABLE I. Fungus Flora in Air and Dust of Bronchial Asthma Patients' Room.

Classification	Air			Dust				Total	%
	A	B	C	A	B	C	D		
Penicillium	4	2	11	3	3	10	7	40	33.3
Aspergillus	1	4	7			6	3	21	17.5
Cladosporium				1	1	1	4	7	5.8
Chaetomium			5			1		6	5.0
Trichoderma			3	1			2	6	5.0
Alternaria	1		1	1			1	4	3.3
Fusarium							3	3	2.5
Oospora						1	2	3	2.5
Botrytis		1					1	2	1.7
Mucor	1			1				2	1.7
Cephalothecium					1			1	.8
Helminthosporium							1	1	"
Scopulariopsis						1		1	"
Spicaria						1		1	"
Macrosporium			1					1	"
Torura							1	1	"
Unclassified	2	3		2	2	5	6	20	16.7
Total	9	7	31	9	7	26			
	—47—			—42— 31				120	

A, patient's room; B, patient's adjoining room; C, closet of patient's room; D, from a cat, blanket, etc., belonging to patient.

cal conclusion can be drawn from the above results as to the presence or absence of any actual active substance or degree of activity involved. However, it may be assumed that some contracting or relaxing substance is contained in the test material which caused intense contraction or relaxation of the tracheal muscle. Seven culture broths caused intense contraction of the tracheal muscle

preparation, 16 moderate contraction, 31 slight contraction, 3 intense relaxation, 3 moderate relaxation, and 6 slight relaxation (Table II). The other 54 culture broths produced no effect. From these culture broths, fungus metabolites were extracted and chemically purified and are now being investigated for their effect on tracheal muscle.

3) *Action on tracheal muscle of metabolites produced by species Oospora.* Among the Oospora species a fungus was found, the culture broth of which strongly contracted the tracheal muscle (Table II). As the fungus was morphologically found to be a new strain, it was designated *Oospora astringens* Yamamoto(2). From metabolites of this fungus were isolated 3 isocoumarine derivatives, which were designated by the authors Oosponol(3) (estimated structure: 3-hydroxymethyl-keton-8-hydroxyisocoumarine), Oospolactone (estimated structure: 3,4 dimethyl-8-hydroxyisocoumarine) and K-I (provisional designation), respectively.

Ten $\mu\text{g/ml}$ of Oosponol contracted the tracheal muscle noticeably in the *in vitro* experiment(4,5). Its effect was a little stronger than that of 100 $\mu\text{g/ml}$ of BaCl_2 . The contraction produced by Oosponol was not antagonized by atropine, antihistaminics, or antiserotonics, and only slightly by washing.

TABLE II. Effect of Culture Broths of Air-Borne Fungi on Tracheal Muscle Preparation.

Classification	No. of species	No effect	Contraction			Relaxation		
			+	++	+++	+	++	+++
Penicillium	40	17	12	2	1	4	3	1
Aspergillus	21	6	6	5	3	1		
Cladosporium	7	6	1					
Chaetomium	6	2	3	1				
Trichoderma	6	2	1	1	1	1		
Alternaria	4	2	2					
Fusarium	3	2			1			
Oospora	3	2			1			
Botrytis	2	2						
Mucor	2	1	1					
Cephalothecium	1	1						
Helminthosporium	1			1				
Scopulariopsis	1	1						
Spicaria	1	1						
Macrosporium	1	1						
Torura	1			1				
Unclassified	20	8	5	5				2
Total	120	54	31	16	7	6	3	3

+, Slight contraction or relaxation; ++, moderate contraction or relaxation; +++, intense contraction (as great as that induced with acetylcholine 1 $\mu\text{g/ml}$) or relaxation.

Oosponol-induced contraction could be relaxed with 0.05 $\mu\text{g/ml}$ of epinephrine, 0.5 $\mu\text{g/ml}$ of papaverine, and 10 $\mu\text{g/ml}$ of theophylline. From this it can be seen that the action of Oosponol on the tracheal muscle is similar to that of barium chloride, but differs as follows:

a) Tracheal muscle preparation contracted with Oosponol can hardly be relaxed by washing. b) With repeated application of Oosponol, the height of contraction on the kymograph is gradually lowered. c) The height of contraction by Oosponol is greatest at 50 $\mu\text{g/ml}$, and conversely declines in higher concentrations. d) Oosponol has no contracting action on small intestine preparations.

Oospolactone could not be tested by the method used in the present experiments, since it is insoluble in water. A substance provisionally designated as K-I showed weak relaxing action in tracheal muscle and intestinal preparations. This was investigated for its effect in relaxing contractions induced with acetylcholine, histamine, serotonin, BaCl_2 , antigen and Oosponol. In a dose of 50 $\mu\text{g/ml}$ it was found to relax contractions induced with serotonin, BaCl_2 , Oosponol or antigen. To relax contraction induced with histamine, 100 $\mu\text{g/ml}$ of K-I was necessary. K-I could not relax contraction induced with acetylcholine.

4) *Action of patulin on tracheal muscle preparation.* As it became clear from the above experiments that some metabolic products of fungi contained substances which produced intense contraction of the tracheal muscle preparation, experiments were performed on various known fungus metabolites other than Oosponol, and it was found that patulin, a metabolite of species penicillium and aspergillus, contracted the tracheal muscle noticeably. The tracheal muscle preparation was contracted with 5 $\mu\text{g/ml}$ of patulin more conspicuously than with 100 $\mu\text{g/ml}$ of barium chloride. Contraction produced with patulin could not be relaxed by atropine, antihistaminic or antiserotonic agents. Epinephrine, papaverine and theophylline would

produce relaxation. Washing was not effective in producing relaxation. With repeated application, contraction lessened sharply. The tracheal muscle contracting action of patulin closely resembled that of Oosponol.

Summary. 1 From a room of 4 bronchial asthma patients were isolated and cultured 120 strains of fungi, including penicillium, aspergillus, cladosporium, chaetomium, etc. Among them was found a new strain of Oospora, which was designated *Oospora astringenes* Yamamoto. 2 Culture broths of these 120 strains were investigated as to their action on the tracheal muscle preparation from the guinea pig. A marked contracting action was observed for culture broths of *Oospora astringenes*, one species of penicillium, 3 species of aspergillus, and one species each of trichoderma and fusarium. Culture broths of one species of penicillium and 2 unclassified strains relaxed the tracheal muscle remarkably. 3 Three new isocoumarin derivatives were discovered in the metabolites of *Oospora astringenes*. Their chemical properties were clearly identified, and they were designated Oosponol, Oospolactone and K-I, respectively. Oosponol strongly contracted the tracheal muscle preparation in the *in vitro* experiment. This action is considered to be different from that of the known tracheal muscle contracting agents. K-I relaxed contraction of the tracheal muscle produced with serotonin, barium chloride, Oosponol, antigen, histamine, but not contraction produced with acetylcholine. 4 Patulin, one of the fungus metabolites, also strongly contracted the tracheal muscle. Its mode of action resembled that of Oosponol.

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Received May 23, 1963. P.S.E.B.M., 1963, v113.