Report on **Garlic & Oil of Garlic** 11/21/72

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REPORT

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GARLIC

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and

OIL OF GARLIC

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200 C Street, SW
Washington, D.C. 20204

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Project Manager

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Laboratory No. 1216

Howard Feinman, Director
Biological Sciences

Food and Drug Research Laboratories
INCORPORATED

Maurice Avenue at 58th Street
Maspeth, New York 11378

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GARLIC and OIL OF GARLIC

(Summary: Toxicological Information)

Very few toxicological investigations of garlic and/or of garlic oil are to be found in the literature; this may be due, at least in the case of the oil, to its obnoxious odor. In the main, the toxicological observations reported have been made in the course of studies aimed at establishing the basis for the historically alleged curative power of the foodstuff.

Powdered garlic was included as one of the foods in a study to determine the role of food in the control of epidemic disease. A semi-synthetic diet containing the foodstuff under investigation was fed to white rats which were observed through 2 generations. Garlic powder caused a slight, but statistically insignificant, lowering of the Hb concentration and red cell count of rats. The second generation of animals fed 5 % garlic powder were sterile. The incidence of lung congestion which appeared spontaneously in a large percentage of stock animals was not influenced by the garlic. (133)

Inhalation of an aerosol of garlic juice diluted 1:3 with water and containing euspiran (6 drops per 2 ml of the juice) was found to be of benefit to patients suffering from bronchial asthma. In 6 out of 21 patients remission of the asthmatic condition was attained. (77)

In contrast, inhalation of garlic powder used in the manufacture of sausages caused an asthmatic condition in one of the workers. The symptoms were relieved by the substitution of garlic kernel for powdered garlic. (113)

Garlic (and onion) were found to cause what is described as contact dermatitis of the hands. Raw abstracted substances of these plants seem to be not only allergenic but also to act as primary irritants to the skin. The allergenic extract was found to be water soluble. (38) A more serious allergic reaction was the appearance in a patient of a condition simulating Meniere's Syndrome (auditory vertigo) as a result of eating garlic (26).

Historically garlic has been used as an effective carminative. X-ray studies of digestion with and without dehydrated garlic included in the diet have shown this to be the result of a calmative action in the gastrointestinal tract by delaying excessive motor activity of the stomach and intestines. (56)

The in-vitro activities of the digestive enzymes are affected by the presence of garlic juice. By itself the juice splits carbohydrates; when added to the various digestive fluids it augments the action of salivary and pancreatic amylases on starch and the action of invertase on sucrose. No effect was detected on the proteolytic and lipolytic action of pancreatin, nor onthe ereptic and lipolytic action of intestinal juice. Peptic activity was found to be impeded by 0.5-2.0 ml of the juice, while 10 ml quantities completely abolished the activity. (47)

Extracts of garlic in vitro also show profound inhibitory effects against sulfhydryl enzymes, which can be explained by the presence of allicin in the extracts. Some enzymes such as triose phosphate dehydrogenase were inhibited by 5×10^{-5} M allicin (308). Inhibition of phosphoglucomutase by an extract from garlic has also been reported (112).

Reports of the antitumor efficacy of garlic continue to appear in the literature. Especially intriguing are those observations made with

garlic given orally for this purpose. For example, the feeding of fresh garlic completely inhibited the development of mammary tumors in female C3H/He mice. Garlic in which the enzyme allinase was destroyed was inactive. Allicin is probably the active principle rather than its precursor alliin. Garlic was ineffective against leukemias of AKR/J mice. (152)

Crude extracts of garlic exerted a marked antimitotic effect on tumor cells. Cytologically, the effects were much like those induced by colchicine, producing blockage of metaphase cells and scattering, as well as abnormal condensation of metaphase chromosomes. (141). In a similar investigation, in vitro exposure of ascites mouse tumor cells to allicin (prepared enzymatically from garlic) completely inhibited cell growth. However, single or multiple injections into mice of up to 30 mg/kg of allicin or of whole garlic extract failed to produce any significant tumor inhibition when tested against both solid and ascites tumors. (61)

Garlic, of the variety Allium cepa, was fed to rats and guinea pigs either raw, cooked, or as various kinds of extracts. In both animals the garlic produced a decrease in red cell count, the decrease being proportional to the amount of garlic fed. Guinea pigs died within 23 days after receiving a diet containing large amounts of garlic. The red count decreased from 5 million to 3.5 million, and variable changes were produced in the white count. (166)

Inclusion of 3% garlic as an additive in the diet of young leghorn chicks improved the growth rate and the feed efficiency as compared with results in the control group. The garlic had no effect on the bacterial flora of the cecum, or on the amount of Vitamin A or thiamine in

the liver (109). On the other hand, in rats receiving a synthetic diet, the administration of garlic even for one day reduced the cecal flora considerably, while in rats receiving stock diet the reduction of cecal flora occurred only after administration of garlic for 5 days. The abnormal increase in the cecal flora of rats receiving a poor rice diet supplemented with red gram dhal or milk was reduced considerably by administering garlic for 5 days. (260)

Oral intake of garlic by the healthy human resulted in an increase of thiamine in feces and in urine. The increase is apparently caused by the synthetic action of intestinal bacteria stimulated by the organic volatile sulfide compounds contained in garlic. Oral ingestion by healthy humans of 10, 100, or 200 mg. garlic oil per day had no influence on the number of erythrocytes in the blood. However, when large doses of garlic oil were injected into cats (100 mg/kg body weight) the animals were brought to an anemic condition. (232)

Garlic oil appears to be an effective anti-helminthic agent against gapeworm. "Almost 100% cures" were realized and "all eggs were sterilized" when infected animals were given oral doses of 0.022 ml of garlic oil in linseed oil. Synthetic allyl sulphide was equally effective. (51)

Other helminths seem to be more resistant to raw garlic. Thus, a man weighing 80 kg and infected with N. americanus and a young dog weighing 10 kg and infected with A. caninum were given known quantities of raw garlic (Allium sativum) mixed with the food for periods of 5 days. Ingestion of 9-14 g daily (54 g total) or 15-21 daily (89 g total) had no evident effect on the output of eggs by Necator. Similarly, ingestion of 7-10 g daily (46 g total) had no evident effect on the rate of egg production by Ancylostoma. However, there was a significant reduction

in the number of larvae of both species developed in fecal cultures made during the period of garlic ingestion. Normal levels were observed within 2 days after termination of intake of garlic. Dead eggs in various stages of development were found in the cultures. (18)

In contrast, oil of garlic, dried garlic, and allyl sulfide among others, administered orally to mice infected with Aspicularis tetraptera showed no activity against this parasite. (255)

Volatile fractions (phytoncides) and tissue juice of garlic showed bacteriocidal action in vitro against a number of microorganisms including E. coli, hemolytic streptococci, and Type 2 pneumococci. 122 patients with acute pneumonia were treated with inhalations of garlic tissue juice, the treatment consisting of 8-40 inhalations over a given period of time, an effective regime being 2 inhalations per day. "A good or satisfactory effect was achieved in 106 patients." (37) The term phytoncides was coined by B. Tokin as a generic appellation for a group of volatile substances (given off by certain plants) which possess bactericidal and "protistocidal" properties. (78)

The in vivo antibacterial action was also demonstrated in patients by treating long unhealed wounds of ulcers with the volatile fraction of garlic phytoncides or with an ointment made with garlic juice. It was observed that necrotic masses and pus were rapidly cleaned from the ulcers and the formulation of granulation tissue was stimulated. (272) In like manner, a 70% solution of garlic tissue juice in distilled water was used for the treatment of 45 patients following surgery for the removal of a phlegmon lesion of the neck. The patients were also injected with this solution intramuscularly or intravenously 3 times daily.

All of the patients were discharged from the clinic in good condition within 9 days after surgery (73).

The reported bacteriostatic and bacteriocidal action of garlic may be due to a substance obtained by alcoholic extraction of garlic residue and identified as allyl disulfide oxide (107).

The indiscriminate intake of garlic fractions is ill-advised (39) since the sulfides of garlic (and of onion) appear to be cumulative poisons to animals, the lethal dose being about 1.2 gm/kg body weight. Because of this poisonous action the use of garlic (particularly fresh garlic) as a therapeutic, bacteriocidal agent is contra-indicated (38). In laboratory animals the cumulative subcutaneous LD_{50} of allicin is 5 mg/kg. The acute subcutaneous toxicity is 50 mg/kg. The intraperitoneal LD_{50} is 20 mg/kg. (204)

Substances present in garlic were found to have a potential effect on the female rat sex hormones. A 2 mg injection (of a 1% ethyl alcohol extract of whole garlic) altered the estrous cycle of ovariectomized rats. The normal cycle of these animals was 2 days estrous and 3 days anestrous while after injection of the garlic extract the cycle was altered to 1 day estrous and 5 days anestrous. Treatment was initiated when the rats had attained a weight of 120 gms. (282)

When rabbits were given 0.015 ml/kg of garlic juice intravenously, a decrease in blood pressure was observed both in the first 5 minutes and in the following hour. The pressure gradually returned to its original level after 2 hours. With a second administration of 0.02 ml. of garlic juice the blood pressure decreased again and remained at that level for 3 hours. No side effects of garlic juice were noted. (149)

Tinctures of garlic diluted with Ringer-Locke solution were per-

fused through isolated ear vessels, kidneys, and rabbit and frog hearts. Garlic was found to exhibit a weak constrictive action on peripheral vessels and to have a definite dilator effect on the beating heart. The effect on heart rhythm was weak but inconsistent, although a definite effect was noted upon the heartbeat amplitude which either increased or decreased according to solution concentration and duration of perfusion. (268)

A non-volatile odorless and water soluble extract of garlic was found to exert an in vivo hypotensive effect and an in vivo and in vitro hypocalcemic effect on mice; the intravenous LD_{50} was 222 mg/kg. The first symptoms of toxicity in these animals are irritability and unstable walking, followed by dyspnea, sluggish movements and finally coma. (248) An antagonist to the hypocalcemic substance may normally be present in whole garlic juice. Thus, after daily administration of garlic juice to guinea pigs, the blood-calcium titer increased becoming 2-3 times the normal value after 3 weeks. After 2 months of this treatment the titer returned to normal. Concomitantly, the calcium content of bone ash increased during the 2 month period, while the P_2O_5 which had diminished in the first 3 weeks, also returned to normal. The calcium content of the teeth increased during the 2-month period of administration and the P_2O_5 titer diminished. (228)

The hypotensive action of garlic extracts was observed in cats receiving i.v. injections of 0.05 g/kg (corresponding to 0.20 g. of garlic). The reduction in blood pressure amounted to 50 mm. In chronic studies in dogs with experimentally induced hypertension, garlic reduced their systolic arterial pressure. In clinical studies on 114 patients having hypertension and atherosclerosis, garlic caused a marked improvement in the systolic (8-33 mm.) and diastolic arterial tension (4-20 mm.) (194)

Later work has shown that alcoholic extracts of whole garlic contain at least 2 pharmacologically active substances. It was observed that one ($CHCl_3$-soluble), had a slight tonic effect on the isolated frog heart, a slight hypertensive effect on etherized cats, and a paralyzing effect on the isolated rabbit intestine. The other, ($CHCl_3$-insoluble), had no effect on the isolated frog heart, a strong hypotensive effect on etherized cats, and a tonic effect on the isolated rabbit intestine. An alcohol extract given by stomach tube to cavies and rats produced no consistent viscera damage. (278)

In contrast to the observed hypotensive effects described above is a report that garlic had no effect on reducing the blood pressures when administered to hypertensive rats. (104)

Another physiologically active substance present in an ethyl ether extract of whole garlic was found to exert a considerable hypoglycemic action when fed to male albino rabbits. It appeared, however, not to be as effective as tolbutamide. (32)

GARLIC and GARLIC OIL

Chemical Information

I. Nomenclature

A. Common names

Garlic

Oil of Garlic

B. Chemical names

(none)

C. Trade names

(none)

D. Chemical Abstracts Services Unique Registry Number

Garlic Oil - MX8000780

Garlic - 977001812

II. Empirical formula

Whole garlic is a mixture of substances with the major components consisting of alliin, allicin, volatile and fatty acids, mucilage, and albumin.(173) Alliin is the precursor of allicin when acted on by the lyoenzyme alliinase. Allicin, but not alliin, has bactericidal properties.(107)

Alliin: $C_6H_{11}O_3NS \cdot 1/2\ H_2O$

Allicin: $C_6H_{10}OS_2$

Column chromatography of whole garlic extracts has led to the identification of a large number of peptides including:

γ-L-Glutamyl-S-propylcysteine (290)

γ-L-Glutamyl-S-allyl-L-cysteine (289)

Garlic oil is obtained by steam distillation of the crushed garlic bulb and consists mainly of disulfides, the principal ones identified as: (107)

Allylpropyl disulfide:	$C_6H_{12}S_2$
Diallyl disulfide:	$C_6H_{10}S_2$
Diallyl trisulfide:	$C_6H_{10}S_3$

Recent work (234) using GLC procedures has identified the following compounds in the volatile fraction of the oil:

Me_2S	$(CH_2 : CH\ CH_2)_2\ S$
Me_2S_2	Me_2S_3

III. Structural formula (107)

Allicin (allyl sulfinyl - allyl sulfide)

$$CH_2 = CH \cdot CH_2 \underset{\underset{O}{\|}}{S} \cdot S \cdot CH_2 \cdot CH = CH_2$$

Alliin

$$CH_2 = CH \cdot CH_2 \cdot \underset{\underset{O}{\|}}{S} \cdot CH_2 \cdot \underset{\underset{NH_2}{|}}{CH} \cdot COOH \cdot 1/2\ H_2O$$

IV. Molecular Weight

Allicin	162.27
Alliin	186.15
Allyl propyldisulfide	148.21
Diallyl disulfide	146.20
Diallyl trisulfide	178.26

V. Specifications

A. Chemical

(none)

B. Food Grade (84a)

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 per cent).

Heavy metals (as Pb). Not more than 40 ppm (0.004 %)

Lead. Not more than 10 ppm (0.001 %)

C. Official Compendia

Food Chemicals Codex

VI. Description

A. General Characteristics (84a)

The volatile oil obtained by steam distillation from the crushed bulbs or cloves of the common garlic plant, Allium sativum L. (Fam. Liliaceae). It is a clear reddish orange liquid, having a strong pungent odor, and flavor characteristic of garlic. It is soluble in most fixed oils and in mineral oil. It may be incompletely soluble in alcohol, It is insoluble in glycerin and in propylene glycol.

B. Physical Properties (71)

Specific gravity at 25°/25°C: 1.040 to 1.090.

(Temperature correction factor from n°/n°C: 0.00058 per °C.)

Refractive Index at 20°C: 1.5590 to 1.5790.

Solubility:

Alcohol: Fairly soluble in alcohol.

Benzyl Benzoate: Soluble in all proportions.

Diethyl Phthalate: Soluble in all proportions.

Fixed Oils: Soluble in all proportions in most fixed oils.

Glycerine: Not soluble.

Mineral Oil: Soluble in all proportions.

Propylene Glycol: Not soluble.

Stability:

Acid: Relatively stable in the presence of weak organic acids.

Alkali: Relatively stable in the presence of weak alkalies.

C. Stability in containers, animal feeds, etc.

Store in full, tight, preferably glass, or aluminum containers in a cool place protected from light. (84a)

VII. Analytical Methods

Alliin: Spectrophotometric estimation following extraction of whole garlic with alcohol (92).

Volatile components: GLC identification of aliphatic mono- and disulfides (224).

Components of whole garlic: Thin-layer chromatographic separation and colorimetric identification. (117)

VIII. Occurrence and levels found in:

A. Plants

Garlic is the compound bulb of Allium sativum L., a perennial plant belonging to the Liliaceae, or Lily family. The compound bulb consists of a number of wedge-shaped bulblets, often called "cloves". The leaves of the garlic plant are long, narrow, flat, and sheath the lower part of the stem. The flowers are purple in color and produced in a cluster at the termination of the stem.

The spice consists of (a) garlic powder, which is the ground product of dehydrated cloves of garlic, and is a creamy-white powder with a strong, persistant aroma and taste; and (b) garlic salt, which is the ground, dehydrated garlic mixed with free-running table salt and sometimes with a small quantity of edible starch to prevent caking. In addition to the above, minced dehydrated garlic is now available. (192a)

B. Animals

(none)

C. Synthetics

(none)

D. Natural inorganic sources

(none)

OIL OF GARLIC

Biological Data

I. Acute toxicity

Animal	Route	Material	LD_{50}(mg/kg)	Ref.
*	cumulative s. c.	allicin	5	204
*	acute s. c.	allicin	50	204
*	i. p.	allicin	20	204
mice	i. v.	"a water soluble extract of garlic"	222	248

* "Laboratory animals"

II. Short-Term Studies

Garlic, of the variety Allium cepa, was fed to rats and guinea pigs as raw garlic, cooked, or various kinds of extracts. In both animals the garlic produced a decrease in red cell count; the decrease was proportional to the amount of garlic fed. Guinea pigs died within 23 days after starting the animals on a diet containing large amounts of garlic and the red count decreased from 5 million to 3.5 million. Variable changes were produced in the white count. (166)

Inclusion of 3% garlic as an additive in the diet of young leghorn chicks improved the growth rate and the feed efficiency as compared with results in the control group. The garlic had no effect on the bactericidal flora of the cecum or on the amount of Vitamin A or thiamine in the liver (109). On the other hand, in rats receiving a synthetic diet, the administration of garlic even for one day reduced the cecal flora considerably, while in rats receiving a stock diet; the reduction of cecal flora occurred only after administration of garlic for 5 days. The abnormal increase in the cecal flora of rats receiving a poor rice diet supplemented with red gram dhal or milk was reduced considerably by administering garlic for 5 days. (260)

A non-volatile odorless and water soluble extract of garlic was found to exert an in vivo hypotensive effect and an in vivo and in vitro hypocalcemic effect on mice. The first symptoms of toxicity with these animals were irritability and unstable walking, followed by dyspnea, sluggish movements and finally coma. (248). An antagonist to the hypocalcemic substance may normally be present in whole garlic juice. Thus, after daily administration of garlic juice to guinea pigs the blood-calcium level increased becoming 2-3 times the normal value after 3 weeks. After 2 months of this treatment the level returned to

normal. Concomitantly, the calcium content of bone ash increased during the 2 month period, while the P_2O_5 which had diminished in the first 3 weeks, also returned to normal. The calcium content of the teeth increased during the 2-month period of administration and the P_2O_5 level diminished. (228).

III. Long-Term Studies

Powdered garlic was included as one of the foods in a study to determine the role of food in the control of epidemic disease. A semi-synthetic diet containing the foodstuff under investigation was fed to white rats which were observed through 2 generations. Garlic powder caused a slight, but statistically insignificant, lowering of the Hb concentration and red cell count of rats. The second generation of animals fed 5% garlic powder were sterile. The incidence of lung congestion which appeared spontaneously in a large percent of stock animals was not influenced by the garlic (133).

IV. Special Studies

Garlic oil appears to be an effective anti-helminthic agent against gapeworm. "Almost 100% cures" were realized and "all eggs were sterilized" when infected animals were given oral doses of 0.022 ml. of garlic oil in linseed oil. Synthetic allyl sulphide was equally effective. (51).

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or satisfactory effect was achieved in 106 patients." (37) The term phytoncides was coined by B. Tokin as a generic appellation for a group of volatile substances (given off by certain plants) which possess bactericidal and protistocidal properties. (78)

The *in vivo* antibacterial action was also demonstrated in patients by treating long unhealed wounds of ulcers with the volatile fraction of garlic phytoncides or with an ointment made with garlic juice. It was observed that necrotic masses and pus were rapidly cleaned from the ulcers and the formulation of granulation tissue was stimulated. (272) In like manner, a 70% solution of garlic tissue juice in distilled water was used for the treatment of 45 patients following surgery for the removal of a phlegmon lesion of the neck. The patients were also injected with this solution intramuscularly or intravenously 3 times daily. All of the patients were discharged from the clinic in good condition within 9 days after surgery. (73).

Garlic and extracts of garlic are claimed to be efficacious in the treatment of dysentery, sciatica, and grippe. It also gave positive results in patients suffering from chronic colitis and gastritis. (316). Whole garlic proved to be without effect on tuberculosis-infected animals. Given every day in a dose of 2g. of fresh garlic mixed in food experimentally infected with tuberculosis it was found not to delay or prevent the onset of the disease. However, in concentrations varying from 1.4% to 1.6% a definite inhibitory action was observed on the growth of tubercle bacilli *in vitro* (265).

The folklore belief that eating garlic reduces the blood pressure has a basis in experimental fact. Thus, when rabbits were given 0.015

ml/kg of garlic juice intravenously, a decrease in blood pressure was observed both in the first 5 minutes and in the following hour. The pressure gradually returned to its original level after 2 hours. With a second administration of 0.02 ml. of garlic juice, the blood pressure decreased again and remained at that level for 3 hours. No side effects of garlic juice were noted. (149).

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In clinical studies on 114 patients having hypertension and atherosclerosis, garlic caused a marked improvement in the systolic (8-33 mm.) and diastolic arterial tension (4-20 mm.)(194)

There are at least 2 pharmacologically active substances present in alcoholic extracts of whole garlic. It was observed that one fraction ($CHCl_3$-soluble) had a slight tonic effect on the isolated frog heart, a slight hypertensive effect on etherized cats, and a paralyzing effect on the isolated rabbit intestine. The other, ($CHCl_3$-insoluble), had no effect on the isolated frog heart, a strong hypotensive effect on etherized cats, and a tonic effect on the isolated rabbit intestine. An alcohol extract given by stomach tube to cavies and rats produced no consistent visceral damage. (278)

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Another physiologically active substance present in an ethyl ether

extract of whole garlic was found to exert a considerable hypoglycemic action when fed to male albino rabbits. It appeared, however, not to be as effective as tolbutamide. (32)

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Substances present in garlic were found to have a potential effect on the female rat sex hormones. A 2 mg injection(of a 1% ethyl alcohol extract of whole garlic) altered the estrous cycle of ovariectomized rats. The normal cycle of these animals was 2 days estrous and 3 days anestrous, while after injection of the garlic extract the cycle was altered to 1 day estrous and 5 days anestrous. Treatment was initiated when the rats had attained a weight of 120 gms. (282)

Phytoncides from onion, garlic, mustard, and related plants were observed to rapidly immobilize rat and guinea pig spermatozoa *in vitro*, the killed spermatozoa showing no detectable morphological changes. Attempts to achieve these effects *in vivo* by inhalation of these agents by a test animal (male rat) did not lead to any disturbance of spermatogenesis(276).

Biochemical Aspects

I. Breakdown

(none)

II. Absorption - Distribution

(none)

III. Metabolism and Excretion

(none)

IV. Effects of Enzymes and Other Biochemical Parameters

(none)

V. Drug Interaction

(none)

VI. Consumer Exposure Information

(none)

GARLIC

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Update Addenda:

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11.54.573. Change in some indices of gas exchange in patients with bronchial asthma in the course of medical treatment with inhalations of an aerosol of garlic juice. Fedoseev, G. V., in the symposium: "Phytoncides, their biological role and value in medicine and in the national economy", Kiev, "Nauk. dumka", 1967, 338 - 339.

32 patients (16 - 60 years old) with bronchial asthma (BA) and chronic pneumonia in an exacerbated stage were medically treated with inhalations (10 - 38 for the course) of an aerosol of garlic juice (I), diluted with water (1 : 3), with the addition of euspiran (II; 6 drops to 2 milliliters of I; 21 patients) or without it. In the case of 8 patients, medical treatment with inhalations of I + II brought about a remission of bronchial asthma, improved the indices of lung respiration, and weakened the intensity of the inflammatory process in the lungs. In the remaining patients I + II were ineffective. The application of inhalations of an aerosol of I without II deteriorated the indices of lung respiration and produced coughing and (in part of the patients) attacks of bronchial asthma.

A. G.

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FOOD ALLERGY TO GARLIC WITH MENIERE'S DISEASE SYMPTOMS

J. Benes, K. Prerovsky, L. Rehurek, and F. Kase

Internal Division of the Regional Hospital, Usti nad Labem,

O. Dub, MD, Director

Otorinolaryngological Division of the Regional Hospital, Usi nal Labem,

K. Zeman, MD, Director

Regional Transfusion Station, Usti nad Labem, J. Matousek, MD, Director

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The clinical picture of Meniere's disease is known. However, the problem of the etiopathogenesis of this disease has not been solved up to the present time. There are various views on this problem. One of these views assumes the existence of an allergenic basis for this disease.

The defendants of the allergic theory, such as Williams (7) and Stranbygaard (5) have even defended the view that every attack of the Meniere type is allergically generated. Others, such as Güttich (1), admit the existence of an allergic basis only for frequently recurring paroxysms, whereas Hilger (2) omits any mention of allergy altogether and emphasizes only the vasospasm. Most probably, however, the etiopathogenesis of Meniere's disease consists of a complex of causes which lead to different symptoms and interact in different way in certain cases. In our country, Chladek (4) determined from an analysis of 75 cases of Meniere's disease that in 6.6% of the cases food allergy was the cause of the attacks. Among the foods possessing demonstrated allergenic properties he includes fruit, tomatoes, hazelnuts, potatoes, milk, butter, eggs, cheese, lentils, grits, cocoa, chocolate, veal, pork and lobster.

By our contribution we would like to supplement the above-mentioned list of food allergens and thus to contribute to the knowledge of diseases of the Meniere group which possess an allergenic basis.

By way of an introduction, it should be recalled that definite criteria must be established for a reliable proof of allergenesis. The proof of a food allergy specifically requires that the Meniere's disease attacks be

evoked and repeated by the administration of the same foodstuff. On the other hand, the elimination of such an allergenic substance should also lead to a therapeutic success. An allergic reaction may be substantiated by immunological tests, such as those involving the blood-cell elements, e.g., the leukopenic and thrombopenic indexes.

Case History

Patient O.S., 44 years old, who worked as a manager of a manufacturing department is now again employed as a manager of a training center.

Family history: Hives recurred in all members of the family.

Personal history: Right mastoid at 14 years of age. Acute glomerulonephritis at 25 years of age. The patient himself never had any allergic symptoms.

Present sickness: The first attack of Meniere's disease occurred in 1962. The patient states that he often ate bread with lard and garlic as a snack. He also ate garlic with other foods as well, such as with meats, etc.

Prior to the first attack of Meniere's disease, he sometimes experienced after eating garlic buzzing in the head for 2 to 3 hours, which was sometimes accompanied by sensations of exaggerated heart beat and palpitation as well as the sensation of lack of air. These symptoms were only of short duration and of low intensity. The patient, therefore paid little attention to these symptoms.

On the day of the attack the patient ate supper in a similar fashion. Six hours after supper, he was suddenly awakened by strong vertigo, sustained humming in both ears, and nausea, which later led to vomiting. At the same time he experienced an urge to defecate and he perspired strongly. Because of these symptoms he was admitted into the ear section. The findings of an internal physical examination were, apart from asthenia, within the normal physiological limits. The blood pressure was 130/80, the pulse was 98 per minute and regular. A spontaneous 2nd degree nystagmus was detected.

The otoscopic examination did not reveal any pathology. The blood profile was as follows: Hb 85/13.60 g%, HK 44, leuco 6100; differential count showed

segm. 43, rods 3, eo. 7, baso. 2, and lympho 45.

Urine analysis was normal.

Orientational examination of the jaw joint was within the physiological limits.

X-ray examination of the neck vertebrae did not reveal pathology.

X-ray examination of the ears did not reveal pathology.

EKG curve was within the physiological limits.

Neurological examination revealed the existence of a neurasthenic syndrome.

Eye examination gave normal findings, including the orbit.

With slow improvement, the symptoms persisted for 3 days. On the third day, an audiogram clearly showed the existence of an appreciable perceptual disturbance. Transmission through the air was well as through the bone showed similar loss values.

The audiogram 1 was determined in the intensity plane. The hearing loss in both ears is on the average equal to 20 dB at all frequencies.

The hearing loss experienced by the patient during the first few days disappeared by the end of the 13-day hospitalization period. The therapy included H-infusions, salt-free diet, Theadryl, and Meprobamate.

After discharge, the patent was synptom-free for two whole years. He repeatedly reported only NCA-type symptoms. He took Meprobamate which improved his condition. He avoided garlic as well as garlic-containing foods.

On the day of the next attack that caused him to be again hospitalized, he again ate some garlic. At the same time he rested during the afternoon in question in a garden where garlic was being grown and where the small of garlic was very strong. The attack again awakened the patient during the night about 9 hours after the ingestion of garlic. The clinical profile wholly corresponded to the preceding conditions, including in terms of its duration. All of the repeated examinations were unchanged with respect to the previous findings. Only in the differential blood profile there was no detedable eosinophily. The patient was again discharged after a 20-day hospitalization without any complaints.

During the rest time after this last hospitalization the audiogram was repeated. The resulting values were practically identical for both sides. These audiograms were also similar for transmission through air as well as through the bone.

The audiogram 2 clearly indicates a 4000 Hz decrease for both sides. On the right the decrease is about 20 dB, whereas on the left it is about 25 dB.

During the second hospitalization, the patient was studied allergologically. The tests were carried out with the following results:

Household dust of the general type: strongly positive;

Histamine in 1:1000 dilution: very strongly positive;

Garlic: weakly positive, and

Control: negative.

After the histamine test, no clinical disease symptoms appeared.

We also carried out exposure tests together with the leukopenic tests while observing all dietary restrictions as well as maintaining physical and mental state of rest.

The exposure test was positive. After 8 hours, the same clinical criteria appeared as before. These, however, did not last more than 1 hour.

At the same time we carried out the leukpenic test with the following results:

	Leuko	% eo
Before garlic injection	8700	4
30 min after ingestion	5150	6
60 min after ingestion	4750	6
90 min after ingestion	4900	4

One hour after exposure, the leukoxytes decrease by 3950 relative to the original level which corresponds to 45%.

Since the above incident, the attack recurred again after ingestion of potato soup which contained garlic as well as after the exposure repeated by us during the thrombopenic test. The clinical picture was in agreement with the preceding description.

The results of the thrombopenic tests were as follows:

Before garlic ingestion	175500
15 min after ingestion	94000
30 min after ingestion	104500
60 min after ingestion	126000

The decrease of the thrombocyte number 15 min after the exposure is equal to 46% of the original level.

During this exposure test we also monitored the pulse frequency. Before the start of the study, the patient had a regular pulse count of 68. Forty minutes after the start of the test, the pulse increased up to 104 regular beats per minute which is 36 more than at the start of the test.

During the most recent period, moderate vertigo paroxysms, buzzing in the ears, and hearing decline have recurred even after significant psychic upsets. Simultaneously, the neurotic insidents are becoming worse and are reportedly accompanied by paroxysmal tachycardiums.

In conclusion, it is possible to classigy the above-mentioned case of Meniere's disease as clear-cut case of a food allergy to garlic in the course of our observation. Spontaneous attack occurred for a total of three times after the ingestion of garlic and twice during the exposition tests. The leukopenic and the thrmbopenic tests were positive An equally positive result was also shown by the pulse, and by the frequency test. The patient also has a family predisposition toward allergies The tests using common household dust and histamine in a concentration of 1:1000 are strongly positive. As time goes on, the patient also shows symptoms even after strong emotional upsets. In this case, we are probably dealing with vasospasms due to a neurovegetative lability of the patient.

Summary

We described a Meniere's disease case in which the clinical phenomena are due toa food allergy toward garlic. The allergic basis was confirmed by the leukopenic, thrombopenic, and repeated exposure tests.

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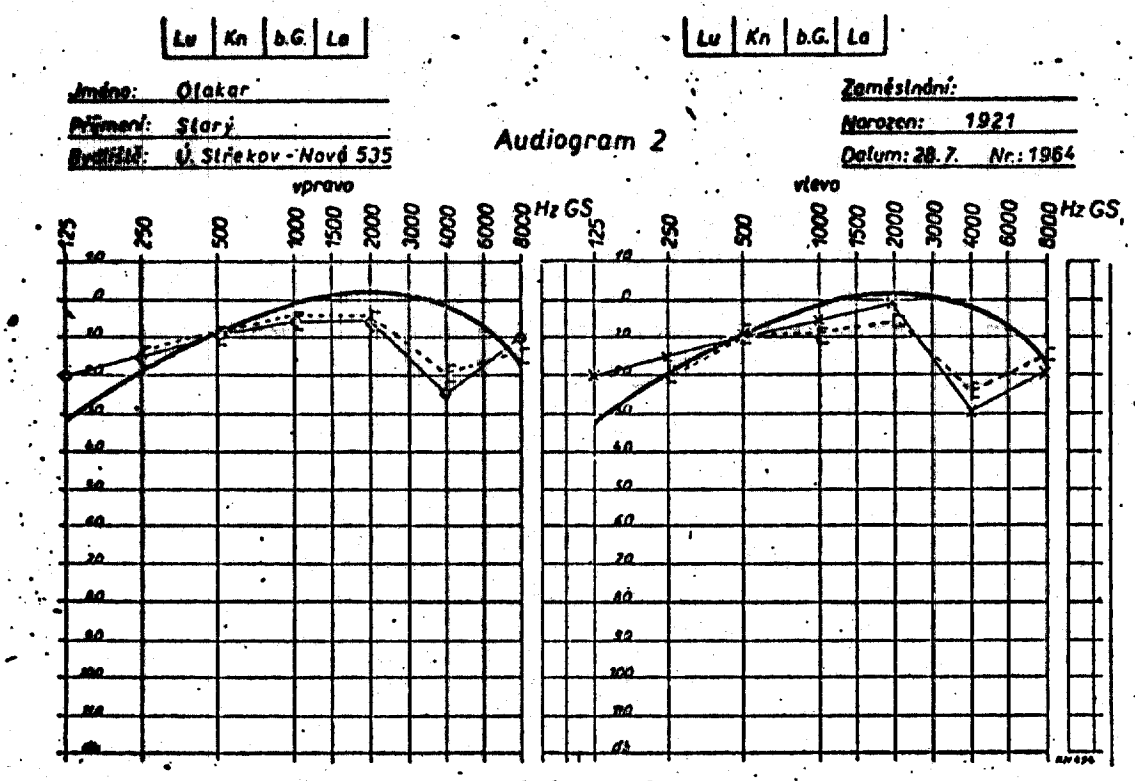

Audiogram 2

Audiogram 2

Key: 1) through 6) same as for Audiogram 1

7) Date: July 28 Year: 1964 *

8) right 9) left

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* The form calls for No.

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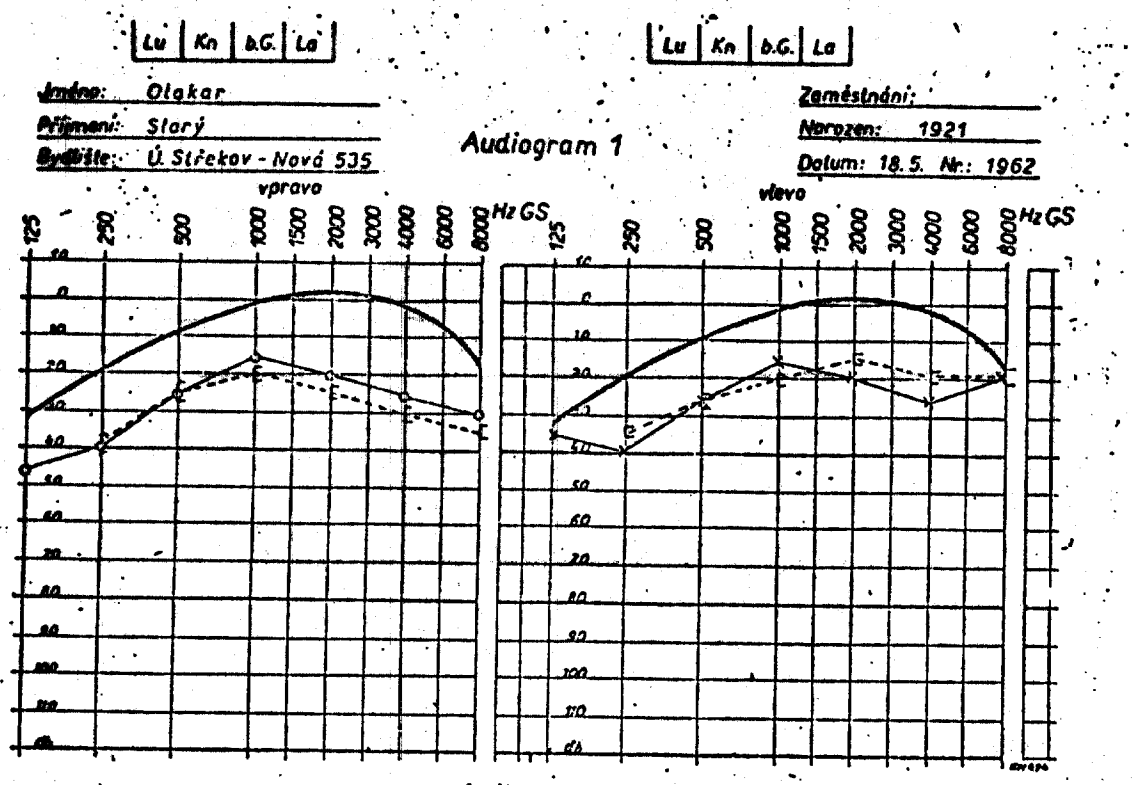

Audiogram 1

Audiogram 1

Key: 1) First name: Otakar 2) Last name: Stary

3) Address: U. Strekov-Nova 535 4) Audiogram 1

5) Employed by: 6) Born: 1921 7) Date: Mary 18

8) right 9) left Year: 1962 *

The Digestive and Zymosthenic Power of Allium Sativum L. in Vitro

By C. Du Chaliot

(From the Institute of Pharmacology, Pharmacognosy, Toxicology and Therapy, University of Palermo, headed by Prof. L. Tocco)

Allium sativum, a liliaceous plant which gros in all inhabited regions of the world, has from antiquity been used by all peoples of the earth for its dietectic and digestive qualities and its many praised medicinal properties, and numerous specialties have been prepared from it and used in therapy. Hipposcrates himself proclaimed Allium sativum an excellent condiment "when one wants to drink and eat well."

The literature on this plant is vast, and eminent therapists throughout the world have become interested in it. But as far as I know, the digestive properties of Allium sativum are more assumed from the use made of it as condiment than from experimental tests.

My work is a study of the digestive power of garlic and its influence on the enzymes that preside over the digestion.

Experiments

In all experiments I have used garlic juice prepared as follows: The bulbs, carefully peeled, were first cut in pieces and then triturated in a mortar, together with glass powder, with an equal weight of well water. I filtered it through gauze and thus obtained a fairly clear liquid of greenish color, of bitter taste and characteristic garlic odor, which I shall in the following call garlic juice for short. The pH of this liquid, tested on the Hellige comparator, was always 6.8 to 7. With this liquid I made three tests.

(1) Garlic juice + substrate under examination; (2) enzyme + substrate; (3) garlic juice + enzyme + substrate.

To avoid the action of germs, I added to the specimens traces of pulverized thymol. Other technical details will be given in the respective experiments.

1. Action on starch and on the amylolytic power of saliva.

To test the influence exerted by garlic juice on the amylolytic activity of

saliva, I measured the time necessary for the color change of the starch solution (soluble starch) to which I had added iodine-iodide solution. I thus determined the time taken for the subsequent transformations into erythrodextrim and achro-dextrin. The saliva was collected, after careful cleaning of the mouth, letting it run into a vessel. The tubes were immersed in a waterbath at 40°C.

Table I

Material under examination	Time of transformation into erythrodextrin	Time of transformation into achrodextrin
Garlic juice, 0.10 ml	min	min
+ Starch solution 1%, 5 ml	65	78
Saliva, 0.5 ml + Starch solution 1%, 5 ml	65	78
Garlic juice, 0.10 ml + Saliva, 0.5 ml + Starch solution 1%, 5 ml	15	25

The results compiled in Table I show that garlic juice alone (also at a concentration of about 0.8%) exerts an amylolytic action on starch and accelerates the action of the saliva on the same starch solution in direct proportion to its percentage (maximum percentage examined by us about 4.4%), as I have deduced from other records.

2. Action on egg albumen and on the proteolytic power of pepsin (1).

To establish the effect of garlic on the proteolytic power of pepsin, I followed the method specified by the Official Pharmacopoeia and, at a pH 1.5, obtained by addition of HCl, I let egg albumen, coagulated by boiling and reduced to small pieces of equal size, be digested in a first flask with garlic juice, in a second flask with pepsin, and in a third flask with garlic juice plus pepsin. The tubes were temperature controlled at 37°C. The progress of the digestion was indicated by the reduction in size of the pieces of albumen.

It is evident from the experiments that garlic juice alone has no effect on the egg albumen; added in minute quantities (1 drop), it has no action; in small quantity (0.50 - 2 ml, or about 0.21 to 0.83%) it hinders but does not cancel

the action of pepsin, while strong doses (10 ml, or about 4.15%) completely abolish the action of the gastric enzyme.

3. Action on the digestive powers of pancreatin. - To investigate how the various activities of pancreatin are influenced, I used fundamentally methods suggested by the Official Pharmacopoeia, adapting them to my purpose. I used pancreatin Merck, available on the market, of which I first tested the activity, which I found reduced, and I therefore used higher percentual quantities than those given by the O.P., preparing a 0.40% solution each time, of which I took the milliliters necessary for my experiments.

a) Action on casein and on the proteolytic activity of pancreatin. In a firs test I let 0.50% casein solution be digested with garlic juice, in a second test with pancreatin, and in a third test with garlic juice and pancreatin. I brough the pH of the specimens to 8 by adding NaOH N/10. After 2 hours of temperature control at 37°C, I stopped the proteolysis by adding drops of acetic mixture and compared the quantity of undigested and hence flocculated casein.

I found the following:

(1) 5 ml of the 0.50% casein solution + 0.50 ml of garlic juice + acetic mixture: abundant flocculate, inthe above stated time;

(2) 5 ml of the 0.50% casein solution + 2 ml of the pancreating solution + acetic mixture: less abundant flocculate than in (1);

(3) 5 ml of the 0.50% casein solution + 2 ml of the pancreatin solution + 0.5 ml of garlic juice + acetic mixture: flocculate as in (2).

From this experiment and from the many carried out but not reported I can conclude that garlic juice alone has no action on casein and does not modify the proteolytic activity of pancreatin.

b) Action on soluble starch and on the amylolytic activity of pancreatin. I carried out the tests at a pH 6.8, maintained by addition of monopotassium phosphate and of disodium phosphate, letting soluble 1% starch solution digest in the first tube with garlic juice, in the second tube with pancreatin solution, and in the third tube with garlic juice plus pancreatin solution,and noting the time necessary for the color change of the starch solution, to which I had added

iodine-iodide solution, indicating the transition to erythrodextrin and to achroextrin.

Table II

Material under examination	Time of transformation into erythrodextrin	Time of transformation into achrodextrin
Starch solution 1%, 10 ml + Garlic juice, 0.25 ml	min 60	min 85
Starch solution 1%, 10 ml + Pancreatin solution, 2 ml	65	90
Starch solution 1%, 10 ml + Garlic juice, 0.25 ml + Pancreatin solution, 2 ml	30	45

As is seen from this record and as I conclude from the other experiments not reported, garlic juice alone breaks down the starches and favors the action of the pancreatin in direct proportion to its quantity (percentages tested by us 1-2%).

c) Action on olife oil and on the lipolytic activity of pancreatin. The tests were carried out at pH 8.9, obtained by adding a molar solution of ammonium chloride and a molar solution of ammonia. I let the oil, netralized as indicated in the O.P., be digested in a first tube with garlic juice, in a second with pancreatin solution, and in a third tube with garlic juice plus pancreatin solution. After 2 hours at 37°C, I quantitatively analyzed the fatty acid formed alcohol solution N/10 of NaOH, using thymolphthalein as indicator.

Table III

Material under examination		NaOH N/10 used in the titration
Olive oil + Garlic juice	5 ml 0.50 ml	ml 0.60
Olive oil + Pancreatin solution	5 ml 4 "	9.8
Olive oil + Garlic juice + Pancreatin soln.	5 ml 0.50 " 4 "	9.7

It is evident from this record that garlic juice has no action on olive oil and does not modify the lipolytic activity of pancreatin; the same may be concluded from other records, which are omitted for the sake of conciseness, even when the dose of garlic juice is increased.

(4) Action on the enzymatic activity of the enteric juice. - I collected the enteric juice needed for my research from a dog on which a Thiry-Vella fistula had been performed. The animal was a male dog of an initial weight of about 6 kg, which while subjected to experimentation remained in excellent health, even gaining weight. The collection of enteric juice was made one hour after a mixed meal. Following irrigation of the loop with 100 ml of a 30% salt solution, I collected the juice which ran from the loop within the first fifteen minutes. It contained bits of tissue, traces of blood and mucus. Befure using it, I triturated it in a mortar together with glass dust, then added an equal volume of physiological solution and then filtered it through gauze. In these experiments I followed in general lines the techniques described by the usual treatises, adapting them to my purposes.

a) Action on saccharose and on the invertase of the enteric juice. I used a commercial saccharose solution of 2.50% and carried out a first test with garlic juice, a second test with enteric juice, and a third test with garlic juice plus enteric juice.

Before making the experiment, I tested the reducing power of the garlic juice and of the sugar, which proved to be zero, while after 5 hours at 37°C there occurred the reduction shown in the following table. The tests were made at pH 7.5. I assayed the reducing power of the samples with the Hagedorn-Jensen method.

Table IV.

Material under examination	Glucose
Saccharose solution 2.5%, 5 ml	0.03 g
Garlic juice, 0.50 ml	0.18

Saccharose solution 2.5%, 5 ml + Garlic juice, 0.50 ml	0.50
Saccharose solution 2.5%, 5 ml + enteric juice, 1 ml	1.20
Saccharose solution 2.5%, 5 ml + Garlic juice, 0.50 ml + Enteric juice, 1 ml	3.60

It is seen from this record that garlic juice alone hydrolyzes saccharose; added to enteric juice it increases the action of the invertase. From others, not reported, I conclude that by itself it hydrolyzes the saccharose in direct proportion to the percentage (percentages tested about 0.8 - 4.1%), and that added to the enteric juice it increases the action of the invertase again in proportion to the quantity added.

b) Action on peptone and on the ereptic power of the enteric juice. I let commercial peptone solution 10%, pH 7.5, obtained by addition of sodium carbonate solution, be digested in a first tube with garlic juice, in a second tube with enteric juice, and in a third tube with garlic juice plus enteric juice. After twelve hours at 37°C, I determined by Störensen's method the quantity of substances formol-titratable with a solution N/10 of NaOH.

Table V.

Material under examination	NaOH N/10 used in the titration
Peptone solution 10%, 10 ml + Garlic juice, 0.25 ml	0.06 ml
Peptone solution 10%, 10 ml + Enteric juice, 1 ml	8.7
Peptone solution 10%, 10 ml + Garlic juice, 0.25 ml + Enteric juice, 1 ml	8.8

This experiment shows that garlic juice has no action on peptone and does not modify the action of the ereptic power of the enteric juice. From the other records, not reported, it is seen that even when increasing the dose,

the garlic juice has no action either on the peptone or on the ereptic power.

c) Action on the lipolytic power of the enteric juice. In a first test tube I let neutral oil be digested with garlic juice, in a second tube, neutral oil with enteric juice, and in a third tube, neutral oil with garlic juice plus enteric juice, at pH 7.5 obtained with solutions of ammonia and ammonium chloride. After twelve hourse at 37°C, I titrated the fatty acid liberated with alcohol solution N/10 of NaOH.

Table VI.

Material under examination	NaOH N/10 used in the titration
Neutral oil, 5 ml + Garlic juice, 0.10 ml	0.15 ml
Neutral oil, 5 ml + Enteric juice, 1 ml	2
Neutral oil, 5 ml + Garlic juice, 0.10 ml + Enteric juice, 1 ml	2.1

It follows from this experiment that garlic juice has no action on the lipolytic power of the enteric juice, and from others not reported it follows that also when increasing the dose there is no action.

Conclusions

The digestive properties of Allium sativum L. are more assumed from the use made of it as condiment than from experimental tests; this is why the Author has studied the digestive power of garlic and the influence which it exerts on the enzymes that preside over the digestion.

From his experiments he draws the following conclusions.

Garlic juice alone breaks down the carbohydrates, and added to the digestive juices it increases the action of the salivary enzyme and of pancreatin on starch and of invertase on saccharose, but it does not influence the proteolytic and lipolytic action of pancreatin, or the ereptic and lipolytic action of the enteric juice. At very small doses it has no action, at medium

doses it hinders and at strong doses it annuls the action of pepsin.

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(1) The pepsin used was from C. Erba at strength 1/3000; we had preserved it for some time and in my experiments it proved of lower strength.

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to return to the normal values if the experiment is continued for a long time, while the aforesaid changes in the formula persist. The number of the white cells and the total value remain within physiological limits.

Guinea pigs.- In the guinea pig, the administration of A.c., raw as well as cooked, whether of dry extract or fluid, led to a certain numerical disorder of the erythrocytes. In the first phase of the experiment, in fact, the animals react with alternating wide oscillations around the normal values; then a state of adaptation seems to establish itself and the erythrocytes become stabilized on a number slightly below the starting values. The number of white cells and the total value remain normal, while the formula shows a marked lymphocytosis with neutropenia and moderate eosinophilia.

The results obtained by us - relatively modest as compared with those stated by the above authors on rats treated with equal doses of A.c. or even lower doses than those used by us - made us suspect that the quantity of active principle contained in our plant was lower than that used by them.

In fact, when administering to a guinea pig the same quality of A.c. but in doses equal to 54 per kg b.w. per day, there occured a rapid and progressive reduction of the red cells which, on the 23rd day, went from the initial 5,000,000 to 3,500,000 with subequent death of the animal.

In this case of relatively acute progression, the leukocyte formula showed much slighter and more inconstant modifications.

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(Translated by Carl Demrick Associates,Inc/LH/t)

Effect of the Allium Diet on the Synthesis of Vitamin B_1 in the Intestinal Tract (1).

Test on Dietary Intake of the Allium Plants

By Zentatsu Sato (Director, Juko Katsura Professor), Katsura Internal Medicine Faculty, School of Medicine, University of Niigata.

Introduction

From the standpoint of the fact that the B_1 which is synthesized by intestinal bacteria supplements in part the amount of B_1 required by living bodies, thereby playing an important role in the living mechanisms, it is extremely important in terms of true nutritional value judgements to examine the effects of the various components of food intake upon the synthesis of intestinal B_1.

Whipple, Church (1), Guerrant, Dutcher et al. (3) have discussed the effect of the components of dietary intakes (food) upon the synthesis of B_1 in the intestinal tract. In recent years, Williamson and Parsons I3) made public a report in which they pointed out the fact that the fibers promote the synthesis of B_1 in the intestines. However, the effect of fibers does not seem to be so marked.

According to Najjar and Holt (4), it is stated that, amongst the carbohydrates, rice and sucrose reduce the synthesis of B_1 in the intestines but that dextrose (grape sugar) promotes the same. If it is established that rice does reduce the synthesis of B_1 in the intestines, it would pose a serious problem to the Oriental peoples whose staple food is rice as pointed out by the author. If there is such foodstuff as will give an extremely favorable effect in terms of nutritional science upon the synthesis of intestinal B_1 in the digestive organ so as to be absorbed into and utilized by the living bodies, I would call it the intestinal B_1 synthesis promotion food. Since nothing has been reported in the past on the intestinal B_1 synthesis promotion food, I felt it necessary to investigate it.

As is well known, the structural formula of B_1 is based on the methylene

combination of a pyrimidine nucleus (which will hereafter be abbreviated as the P nucleus) and a thiazole nucleus (which will hereafter be abbreviated as the T nucleus). Even though the P nucleus exists in the nucleonic acids which are contained in the cell composition in the food which is used by us every day, the P nucleus does not exist in nature or, for that matter, in food in its independent state at all. Since it is a known fact that micro-organisms easily synthesize B_1 from the P nucleus and the T nucleus, it can be easily understood that the synthesis of intestinal B_1 can be carried out to a marked extent if it is at all possible to introduce the T nucleus, as it is, into the intestines. Since there does not exist any food which contains the T nucleus as it is, as was stated earlier, therefore, I hit upon organic S for the T nucleus. Among these organic S compounds, I selected diallyl sulfide as a sulfide having a structural formula which is as similar to the T nucleus as possible. Assuming that the T nucleus may be created if N is introduced into it, with the result that an intramolecular conversion takes place and that the ring is closed, I thought that B_1 might be synthesized by intestinal bacteria from it and from the P nucleus. I therefore resorted to the allaceous plants which contain organic sulfides.

When allaceous plants were fed to humans as auxiliary diet (refer to the attached table, these garlic plants will hereafter be referred to as allium), there was found a marked increase in the content of B_1 in the excretions as had been expected (both in feces and urines). In other words, I discovered the fact that allium is a remarkable B_1 resource having a new significance as foodstuff promoting the synthesis of intestinal B_1.

The use of allium as food by humans dates back to literature 4500 years before Christ, which described the fact that the cost of garlics and leeks by workers engaged in the construction of great pyramids at Chizcho reached a tremendous sum. It is not difficult to imagine how much the working ability of the hard laborers in the tropical region, who consumed a great volume of B_1, was raised by the regular intake of allium. In ancient Egypt, approxi-

mately 3400 years before Christ, the garlic, onions and leeks were raised enthusiastically and were consumed avidly by the people. It is also said that allium was favorit food of the people who have left the glorious civilizations of Persia, ancient Greece and Italy, etc. to the present day. It is a well-known fact that garlics and leeks are taken regularly by peoples in China, Mongolia and Korea. It is not difficult to understand why these people, who take low-level nutritional food, can well withstand heavy labor.

Table 1 (p. 185) Allium plants.

Name	Scientific name	Evaporative oil portion (name of the researchers)
1. Leeks	A. odorum L.	
2. "Hime" leek	A. monanthum maxim.	
3. Wild rocambole	A. nipponicum Franch. et Sav.	
4. "Sutego" rocambole	A. inutile Makino	
5. Onions	A. Cepa L.	Allylpropyldisulfid Allylpropylpolysulfid (Semmler 1892)
6. Scallions	A. Bakeri Regel	
7. Wild scallions	A. japonicum Regel	
8. Stone-leeks ("Negi").	A. Fistulosum L.	Diallylsulfid (Wertheim 1844-45)
9. Chive Garlic	A. Ledebourianum Schult	
10. White Horse Chive Garlic	A. Schoenoprasum I.	(Allylpropyldisulfid (Diallyldisulfid (Diallyltrisulfid
11. Garlic	A. sativum L.	((Semmler 1892 Karai 1926)
12. "Tyoja" garlic	A. Victorialis L.	(Diallylsulfid ((Wertheim Karai)

Garlic has been used in the treatment of communicable diseases and lung tuberculosis because of its medicinal value in terms of germicidal and stimulative effects. It has also been used amongst the lay people as an extra-energy agent and as a medicine for the removal of worms.

As has been described above, allium has been used widely since olden days as food. As will be clear later, it is clear that allium serves greatly in

the synthesis of intestinal B_1 in the study described in this report. It has become necessary to further raise the rating of allium as food which has been a favorite food since olden days throughout the world as an important food because of its medicinal value.

As Report No. 1, this paper will describe the effect of allium taken as auxiliary food upon the synthesis of intestinal B_1.

Experimental Method

Healthy people were selected as the objects of this test. During the duration of the test, these people were made to take the meals consisting of almost the same menus (2300 to 2500 calories, fat in the range between 30 and 25 grams, protein in the range between 100 and 125 grams, carbohydrates in the range between 390 and 405 grams, and P_1 in the range between 1.4 and 1.7 milligrams). During this period, these people were given allium as auxiliary food. In this manner, the increase or decrease in the amount of B_1 contained in the excretions (feces and urine) every day was measured in accordance with the thiochrome process.

The increase or decrease in the B_1 content in feces and urine was shown as a percentage increase or decrease by comparing the average amount of B_1 discharged a day before the intake of allium and the average amount of discharge of B_1 a day after the intake of allium.

(Figure 1 on p. 185.) Increase or decrease in B_1 contained in feces and urine when onions were taken (during the period between September 11 through September 20, 1950). Person Examined: Mi( )ko, 31 years, F.

a. Urine. b. Feces. c. B_1 concentration(gamma per cent). d. 370 grams of onions per day. e. Number of days passed. f. Urine. g. Feces. h. Total B_1 volume(gamma). i. 370 grams of onion per day. j. Number of days passed.)

Experimental Results

(I) Onion Intake Test.

The period of the test ran from September 11, 1950 through September 20, (1950). The person examined was Mi( )ko Sa( ), a housewife, 31 years.

Three hundred seventy (370) grams of onions were cooked with butter for a

short period of time, with soy sauce or salt put on same. Otherwise, chicken eggs were added to same. For a period of three days from the fourth day to the sixth day after the start of the experiment, this was given to the subject as auxiliary food at every meal. As a result, Figure 1 was obtained. The B_1 content in feces was shown as the total B_1. In other words, the B_1 content in feces delivered on the fifth day or on the day after onions were taken as auxiliary food showed a marked increase and the B_1 content in urine also showed an increase. There was a rapid return to the normalcy on the eighth day or two days after such feeding was stopped. In this case, the rate of the increase in the B_1 content due to the intake of onions was 108.3 per cent in the case of feces and 42.9 per cent in urine.

(II) Garlic Intake Test:

The period of the test ran from September 22, 1950 through October 1, (1950). The person examined (subject) was Chi( )ko Shira( ), 25 years, a maid.

Fifty five (55) grams of garlic per day was placed in hot ash for a period of from two to three minutes, with the outer skin being unremoved, and, before they were cooled, they were served the subject at every meal. As a result, Figure 2 was obtained. Garlic was fed in the manner described above during the period of three days from the fourth day to the sixth day of the experiment. The garlic feeding was strated and stopped on the seventh day or the day after the start of garlic intake. During the period from the fifth day or the day after the intake of garlic was started to the seventh day when the garlic feeding was stopped, there was a marked increase in the B_1 content in feces and there was an increase in urine, too. A complete return to normalcy was observed on the ninth day. In this case, the rate of the increase in the B_1 content was 103 per cent in feces and 91.8 per cent in urine. Even on the eighth day or two days after garlic intake was stopped, complete normalcy was not returned and there still was a high content in feces and urine.

Figure 2 (p. 186). Increase or decrease in the B_1 content in feces when garlic was taken (during the period from September 22, 1950 through October 1,

1950). Subject: Chi( )ko Sa( ), 25 years, F. a. Urine (gamma %). b. feces (gamma %). c. B_1 concentration (gamma per cent). d. Garlic 55 grams per day. e. Number of days elapsed. f. Feces (gamma per cent). g. urine (gamma per cent). h. Urine (gamma). i. Feces (gamma). j. 55 grams per garlic a day. k. Total B_1 amount (gamma). m. feces. n. Urine. o. Number of days elapsed.)

(III) Stone-leek Intake Test

The period of the test ran from September 22, 1950 through October 1, 1950. The subject (person examined) was Kazu( ) Shin( ), 19 years, a nurse.

Stone-leeks, 280 grams per day, were cut into small pieces and cooked for a short period of time. The subject took this for a period of three days from the fourth day to the sixth day of the experiment. As a result, Table 2 was obtained. In other words, there was observed an increase in the B1 content in both feces and urin during the period of three days from the fifth day of the experiment or the day after stone-leek diet was started to the seventh day of the experiment or the day after the diet was stopped. The rate of the increase in this case was 69.2 per cent in feces and 30.8 per cent in urine.

Table 2 (p. 187) Increase or decrease of B_1 in feces and urine in the case where stone-leeks were eaten (during the period from September 22, 1950 through October 1, 1950). The subject: Kazu( ) Shin( ), 19 years, F.

Number of days elapsed	Total B_1 in Feces		B_1 in urine		Remarks
	gamma %	gamma	gamma %	gamma	
First day	613.6	1104.5	18.0	324	
Second day	642.8	1028.4	16.5	313.5	
Third day	630	1071	19.5	331.5	
Fourth day	645	1032	18.0	306	280 grams of stone leeks
Fifth day	975	1755	22.5	405	280 grams of stone leeks

Sixth day	1050	1785	27.3	042	280 grams of stone leeks.
Seventh day	1050	1880	25.5	433.5	
Eighth day	825	1320	18.0	360	
Ninth day	645	1132	16.5	330	
Tenth day	660	990	16.5	313.5	

(IV) Leek Intake Test:

The period of the test ran from September 22, 1950 through October 1, 1950. The subject was Jun Sa( ), 67 years.

Wild leeks, 280 grams a day, was heated and cooked for an extremely short period of time as in the case of stone leeks. As a result, T ble 3 was obtained. In other words, the subject ate leeks for a period of three days from the fourth day of the experiment to the sixth day of the experiment during the test period of 10 days. During the three days from the fifth day to the seventh day, there was observed a marked increase in the B_1 content in feces and urine. The rate of the increase in this case was 88.5 per cent in feces and 51.3 per cent in urine.

Table 3 on p. 187. Increase or decrease in the B_1 content in feces and urine when leeks were eaten (during the period from September 22, through October 1, 1950). The subject was Jun Sa( ), 67 years, F.

Number of days elapsed	Total B_1 in Feces		B_1 in urine		Remarks
	gamma %	gamma	gamma %	gamma	
First day	600	780	15.0	270	
Second day	630	819	15.0	285	
Third day	642.8	835.6	13.5	270	
Fourth day	630	819	16.5	330	280 grams of leeks
Fifth day	1020	1530	19.5	370.5	280 grams of leeks
Sixth day	1142.8	1485.6	22.5	450	280 grams of leeks

Seventh day	1125	1575	22.5	450
Eighth day	750	1125	18.0	360
Ninth day	750	975	15.0	285
Tenth day	690	897	13.5	283.5

(v) Chive Garlic Intake Test:

The period of the test ran from October 2, 1950 through October 7, 1950. The subject was Chi( )ko Shiro( ), 25 years, a maid.

The subject ate 10 grams per day of chive garlic as it was by using soy bean sauce on the second and the third day of the three-day test period. The chive which was used in this case was the chive which was left as a seed since it was in the middle of the fall and it was difficult to obtain a fresh supply of the same. It was so dry that there was almost no moisture left in it; however, its peculiar smell was still left in it to some extent. As is shown in Tble 4, there was observed an increase in the B_1 content both in feces and urine discharged on the third day or the day following the intake of chive and on the fourth day. In other words, the rate of the increase was 34.3 per cent in the feces and 11.4 per cent in urine.

Table 4 on p. 187) Increase or decrease in the B_1 content in feces and urine in the case where chive was eaten (during the period from October 2, 1950 through October 7, 1950). The subject: Chi( )ko Shiro( ), 25 years, F.

Numbers of days elapsed	Total B_1 in Feces		B_1 in urine		Remarks
	gamma %	gamma	gamma %	gamma	
First day	525	1207.5	12.0	252	
Second day	540	1080	13.5	270	10 grams of chive
Third day	750	1575	13.5	283.5	10 grams of chive
Fourth day	825	1650	15.0	300	
Fifth day	570	1254	12.0	264	
Sixth day	570	1140	15.0	270	

(VI) Wild Rocambole Intake Test

The test period was from October 2, 1950 through October 7, 1950. The subject was Mi( )ko Sa( ), 31 years, a housewife.

Wild rocambole, 135 grams per day, was heated and cooked by heating for as short a period of time as possible and was fed to the subject. The period of the experiment was six days, during which the subject was fed wild rocambole on the second and the third day of the experiment. As a result, Table 5 was obtained. In other words, there was observed some increase in the B_1 contents in feces and urine discharged during the two days including the third day and the fourth day. The rate of the increase was 34.4 per cent in feces and 16.9 per cent in urine.

Table 5 on p. 188.) Increase or decrease in B_1 contained in feces and urine in the case where wild rocambole was eaten (during the period from October 2, 1950 through October 7, 1950. The subject was Mi( )ko Sa( ), 31 years, F.

Number of days elapsed	Total B_1 in Feces		B_1 in urine		Remarks
	gamma %	gamma	gamma %	gamma	
First day	720	864	15.0	300	
Second day	735	876	13.5	270	135 grams of wild rocambole
Third day	900	1170	16.5	330	135 grams of wild rocambole
Fourth day	975	1267.5	18.0	342	
Fifth day	750	900	15.0	285	
Sixth day	735	955.5	15.0	277.5	

(VII) Garlic Water Immersion Leached Solution Intake Test:

The period of the test ran from October 18, 1950 through October 25, 1950. The subject was (1) Mi( )ko Sa( ), 21 years, a housewife, who was made to take 100 cubic centimeters of a five per cent solution per day and (2) Chi( )ko Shiro( ), 25 years, a maid, who was made to take 200 cubic conti-

meters of a five per cent solution per day.

In order to examine the effect of a small amount of garlic without heating, which was considered to be the most effective among all tested in the allium intake test, triturated garlic made into a leached water solution was fed to the subject. In other words, 100 cubic centimeters (five grams of garlic) and 200 cubic centimeters (10 grams of garlic) of a five per cent leached solution prepared by immersing garlic in water were given the subjects. As is shown in Figures 3 and 4, the rate of the increase in B_1 in feces and urine discharged per day in the case where a water leached solution corresponding to five grams of garlic was 85.9 per cent in feces and 22.5 per cent in urine. Also, in the case of a water leached solution corresponding to 10 grams of garlic, the rate of the increase in B_1 per day was 99.3 per cent in feces discharged and 62.6 per cent in urine discharged. This is indicative of the fact that, if heating is not carried out, there is a five to ten-fold increase in the effect upon the synthesis of intestinal B_1 even when the garlic is pulverized as compared with the garlic which was heated for a short period of time.

Figure 3 (p. 183). Increase or decrease in B_1 contained in feces or urine in the case where a garlic water leached solution was eaten (the period from October 18, 1950). (One hundred cubic centimeters of a five per cent solution was taken per day). The subject: mi( )ko Sa( ), 31 years, F. a. Urine (gamma per cent). b. Feces (gamma per cent). c. B_1 concentration (gamma) per cent. d. Garlic water leached solution 100 cubic centimeters. e. Number of days elapsed. f. Feces. (gamma per cent). g. Urine (gamma per cent). h. Feces and urine. i. Total B_1 content (gamma). j. Garlic water leached solution 100 cubic centimeters. k. Feces (gamma). m. Urine (gamma). n. Number of days elapsed.)

(VIII) Long-period Raw Garlic Intake Test:

The period of the test ran from October 5, 1950 through November 21, 1950. The subject was Jun Sa( ), 67 years.

Figure 4 (p. 189). Concentrations of B_1 in feces and urine discharged in the case where a garlic water leached solution was taken (the test started October 18, 1950). (Two hundred cubic centimeters of a five per cent solution was given.) The subject: Chi( )ko Shiro( ), 25 years, F. a. Urine (gamma per cent). b. Urine (gamma per cent). c. B_1 concentration (gamma per cent). d. 200 cubic centimeters of a garlic water leached solution. e. Urine and feces (gamma). f. Total B_1 amount (gamma). g. 200 cubic centimeters of a garlic water leached solution. h. Feces (gamma per cent). i. Urine (gamma per cent). j. Number of days elapsed. k. Feces (gamma). m. Urine (gamma). n. Number of days elapsed.)

Without applying such a mechanical operation as pulverization, five grams of raw garlic a day, which was kept as fresh as possible, was fed to the subject for a long period of six weeks and its effect was examined. As is shown in Figure 5, it was found to be truly a remarkable resource of B_1. In other words, the rate of the increase in the B_1 content in feces discharged perday was 1804 per cent for the three weeks during the first half and 193 per cent for the three weeks during the latter half, the rates of the increase being approximately the same in both.

In addition, the rate of the increase in the B_1 amount discharged in urine per day was 39.8 per cent for the three weeks during the first half and, for the three weeks during the latter half, there was a large increase of 61.5 per cent as compared with the first half.

Figure 5 (p. 189) Increase or decrease in B_1 contained in feces or urine in the case where raw garlic was taken at the rate of five grams per day. (From October 5, 1950 through November 21, 1950). The subject: Jun Sa( ), 68 years, F. Five grams of raw garlic taken per day. a. B_1 discharged in urine per day (gamma). b. Total B_1 in feces (gamma, gamma per cent). c. Feces (gamma). d. Urine (gamma). d. Feces (gamma per cent). f. Number of days elapsed.)

Observations:

As was shown in the experimental result, there is an inevitable increase in B_1 discharged in feces by the intake of allium. It goes without stating that this increase results from an increase in B_1 synthesized by bacteria in the intestinal tract. This is because of the following: When healthy people are used as the subjects as under the conditions of my current experiment, the amount of the intake of B_1 per day even when allium was used as auxiliary food was in the neighborhood of 1.5 milligrams and, according to Mr. Shigemitsu of this Department (5), in the case where the amount of B_1 taken was less than three milligrams per day, the B_1 in feces entirely consists of intestinal bacterium-synthesized B_1. Therefore, it will not be erroneous to state that, in my experiments, the increase in B_1 due to the intake of allium was the increase in intestinal bacterium-synthesized B_1. This is clear from the fact that, as will be shown in a subsequent report, there is no increase in B_1 in feces even when allium is taken by preventing the synthesis of intestinal bacterium B_1 by means of homozurfamine.

Next, whether synthesized B_1 due to the intestinal bacteria has been absorbed or not can be determined by whether there is an increase in B_1 in urine along with an increase in B_1 in feces. It goes without stating that the extent of the increase of B_1 in urine is affected by various conditions. In the case of a healthy person, there is an increase, albeit to different degrees, by the intake of allium, as stated in the section dealing with the Experimental Result.

Among the various forms of allium, garlic and onions showed the highest values in the synthesis of intestinal bacterium B_1 (reference should be made to Figures 1 and 2). Inthe case of stone-leeks, leeks and wild racomboles, the rate of intestinal bacterium B_1 synthesis was clearly lower than in the case of the former two.

This is assumed to be due to the fact that, as compared with the garlic and onions, the heating time required for cooking was long. In the case of wild rocamboles, in particular, the value obtained was the smallest inasmuch as it was difficult to eat the same unless cooking was carried out for a long

period of time.

In the case of chives, old items which hardly contained any moisture were used. As a result of this, these were taken in the raw state. Nevertheless, the value obtained was the lowest among all including the first five. In this manner, the value is considerably affected by the method of cooking allium and it can be said that heating is not desirable.

In the case of garlic and onions, it is believed that the greater the content of a substance which serves the purpose of intestinal B1 synthesis in the composition or a larger evaporative oil content (which will be discussed further in a subsequent report) than in the case of other kinds of allium was instrumental in intestinal B_1 synthesis. As was stated in the Experimental Result section, therefore, it was learned that the taking of a water leached garlic solution, without heating, was the most effective. In the case where raw garlic was taken for a long period of time, synthesized B_1 continues to maintain a high value during the said long period of time. It seems that this is indicative of the fact that this is an excellent food item in terms of nutritional science.

Now, the B_1 content of allium cannot be said to be great, as is admitted widely. Rather, its B_1 content as food is on the low side. Nevertheless, it was clearly found to be effective in intestinal B_1 synthesis to a marked extent as stated above. The fact that this intestinal B_1 synthesis substance of allium is due to the evaporative organic S compound as originally anticipated in concept has been proved in a test-tube experiment which was conducted by Mr. Hideo Terada of this Department (7).

Ten (10) grams of garlic was finely cut and thrown into water and the evaporative oil portion which is the mainstay of the garlic smell or the evaporative organic S compound was evaporated by boiling same by heating for a period of 30 minutes. When this was eaten, there was observed no increase in B_1 contained in feces discharged, nor was there any increase in B_1 contained in urine. (Table 6). This is indicative of the fact that allylsulfide serves in intestinal B_1 synthesis.

Table 6 on p. 190.) Increase or decrease in B_1 contained in feces or urine in the case where 10 grams of garlic was boiled in water for a period of 30 minutes, with the odor being evaporated and escaped therefrom, and given to the subject. The subject: Chi( )ko Shiro( ), 25 years, F.

Number of days elapsed	Total B_1 in feces		Free-type B_1 in feces		B_1 in urine		Remarks
	gamma %	gamma	gamma %	gamma	gamma %	gamma	
First day	600	1200	180	360	15	300	
Second day	525	1103	180	375	15	285	Boiled garlic 10 grams
Third day	570	1140	180	360	15	300	
Fourth day	540	1107	150	308	15	300	
Fifth day	585	1112	160	304	16	320	

Conclusion

The intake of allium plants as food markedly increases the B_1 content in feces and increases the B_1 content in urine. In other words, it was discovered that the allium plants are quite effective in intestinal B_1 synthesis. This is due to the evaporative oil which is a component of these plants and an outline was given as to the concept.

The present study was carried out with a fund allocated from the Science Research Fund of the National Ministry of Education. This is specifically mentioned here to show the gratitude of the author.

At the end, the author expresses his deep thanks to Professor Katsura, who was kind enough to read the manuscript for him. He also expresses his gratitude to Dr. Danji Matsukawa, who directly guided him and who read the manuscript.

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(7) Hideo Terada: Will be published soon in this Journal.

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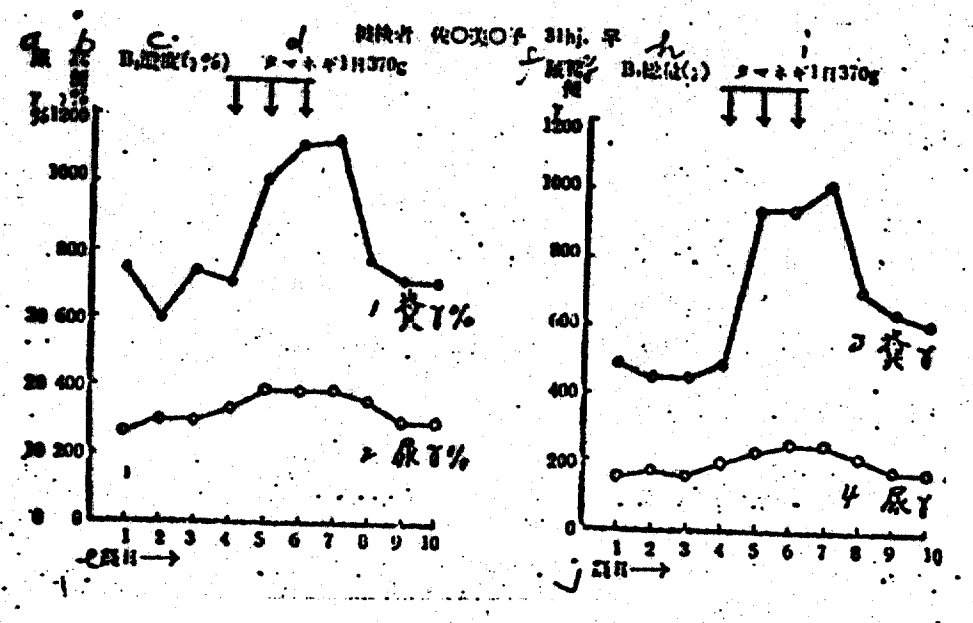


Fig. 1

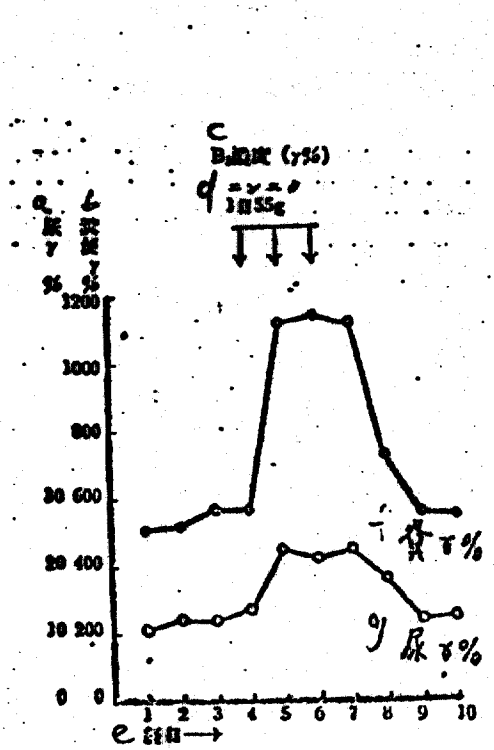
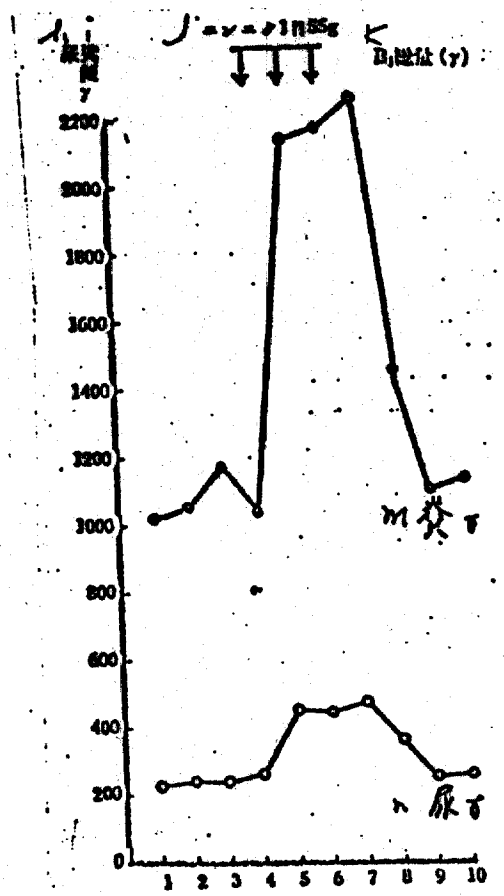


Fig. 2



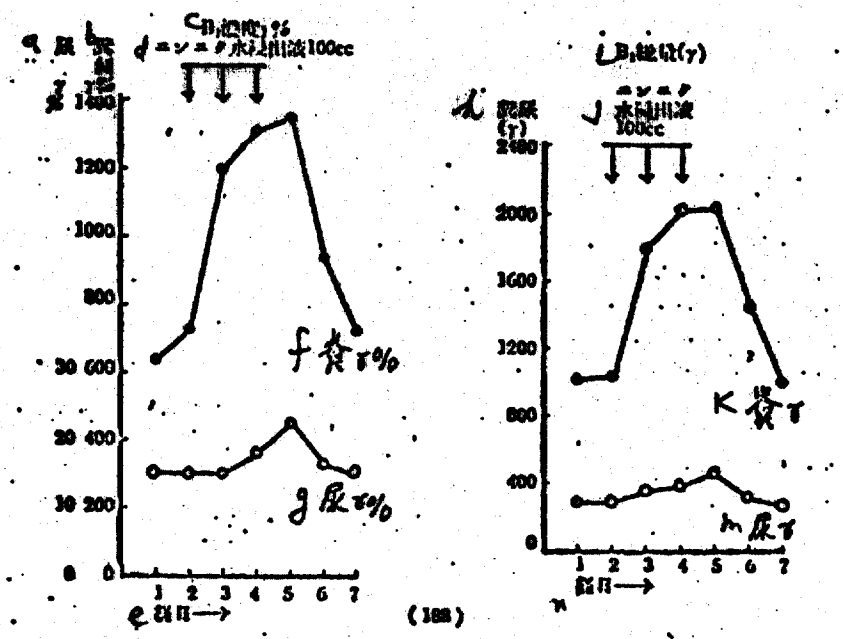


Fig. 3

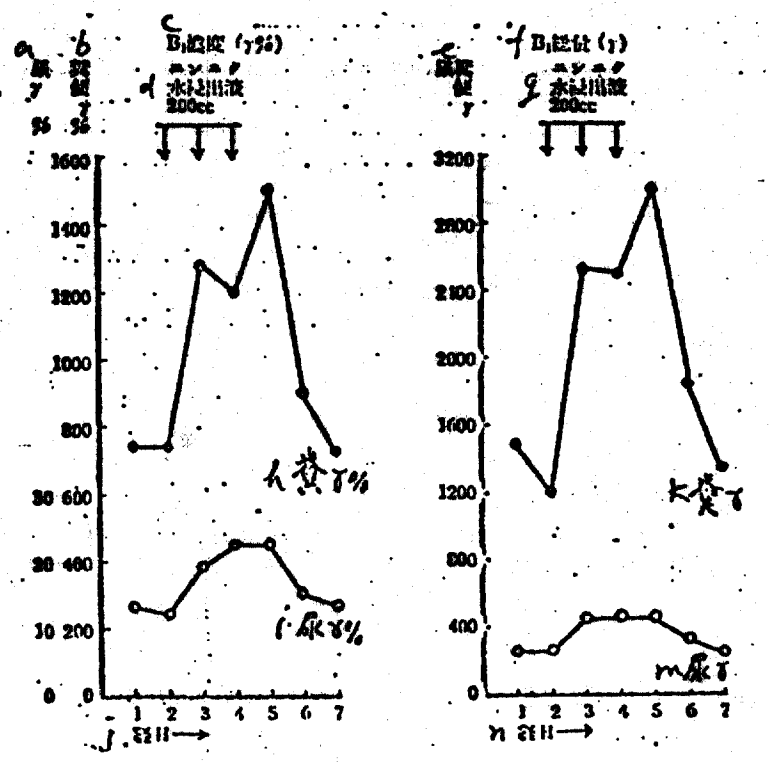


Fig. 4

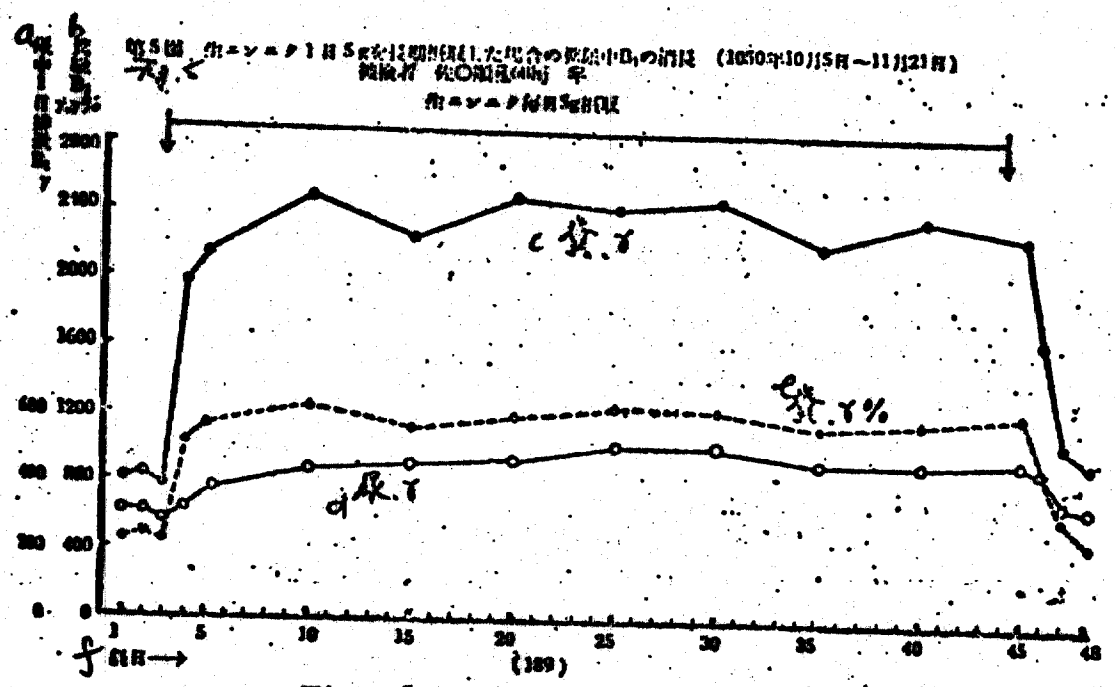


Fig. 5

Medical treatment of patients with various inflammatory diseases of the respiratory organs by means of garlic phytoncides.

Bulatov, P. K., Zlydnikov, D. M., Fedoseev, G. B., Khan-Fimina, V. A., Saraeva, A. N., in the symposium: "Morphology, Physiology and Pathology of the Respiratory Organs", L(eningrad), 1965, 25 - 28.

A study was made, in vitro, of the action of the volatile fractions and of the tissue juice (TJ) of garlic on microorganisms (white, golden and lemon-yellow staphylococcus, hemolytic streptococcus, 2nd-type pneumococcus, intestinal bacteria, Proteidae, Pseudomonas pyocyanea (bacillus), and yeast-like fungi of the Candida type), which are frequently encountered in the sputum in the case of lung diseases. Amounts of 0.1 milliliter of emulsion from a 24-hour culture (500 million microbic bodies in 1 milliliter of a physiological solution) were deposited on the surface of a thick nutritive medium in 3 dishes. Into the 1st dish was introduced 1 gram of ground garlic; in the 2nd one, 0.1 milliliter of garlic tissue juice; the 3rd dish served as a control. From the presence of the sterile zone there was established the bactericidal action (BA) of garlic preparations relative to all the microorganisms. The addition of euspiran (3 drops to 1 milliliter) to the garlic tissue juice did not reduce the bactericidal action. 122 patients with acute pneumonia, exacerbation of chronic pneumonia (CP) and chronic bronchitis were medically treated by means of inhalations of garlic tissue juice diluted in a physiological solution or with a 0.25% solution of novocaine (1 : 3). The course of treatment was 8 - 40 inhalations. A good or satisfactory effect was obtained in the case of 106 patients (86.7%). The addition, to the garlic tissue juice, of antibiotics or sulfamides, exerted no influence on the effect of the medical treatment. Inhalations of garlic by 34 patients with chronic pneumonia complicated by candidiasis of the lungs yielded a positive clinical effect in the case of 26 patients (76.5%); at the same time, a decrease in the amount or a disappearance of the Candida fungi from the sputum was observed in the case of 16 patients (47%).

A. Gladkikh

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(From the Pharmacological Institute of the Academy of Military Medicine, Leningrad (Director: Prof. S. W. Anichkov*))

Direct Action of Garlic (Allium sativum) on the Heart and Vascular System

An experimental investigation

By Dr. W. A. Svechnikov and Dr. S. W. Bekhtereva

Text includes 14 illustrations

(Received on December 15, 1930)

A number of articles devoted to the healing power of garlic have recently appeared in the medical press. Still more frequently, garlic is used by the patients themselves without any medical prescription, and it may be said without exaggeration that there are few sclerotics who are not at present using garlic preparations.

The favorable effect of garlic in heart diseases, preferably in sclerosis of the heart and attacks of angina pectoris, was noted by Schlesinger (1), S. May (2), Schwahn (3), Pouillard (4) and others, and in diseases of the gastro-intestinal tract by Noether (5), Roos (6), Tilger (7), Bonem (8), Bahnem (9), Plaut (10) and others.

This prompted us to take up in our clinic the study of the effect of garlic on a number of internal diseases. The literature accessible to us offered us little basis for the use of garlic. Few indications on the experimental testing of its effect on the gastro-intestinal tract are contained in the papers of Noether, Bahem and Bonem. Vincent (12), Sunceri (13) and Loeper, Debray and Cheilly-Bert (14) have studied the effect of garlic on the circulation in the whole animal organism. But there are absolutely no publications on research of its effect on the cardiovascular system of isolated organs.

This fact gave us the idea to undertake an experimental study of the effect of garlic on the heart, vascular system and digestive apparatus prior to its clinical testing. We presumed that such a study would serve as a brief introduction to that of the effect of garlic on the sick human organism. However,

already after the first experiments we realized the difficulties in exploring the effect of garlic, and that a full understanding required numerous experiments, the result of which constitutes the present paper. The second part of our work - the experimental exploration of the effect of garlic on the digestive apparatus - is at present being carried out separately.

For our experiments we used a garlic tincture of 40 parts garlic to 100 parts alcohol 95%.

In Zörnig's article we found data on the composition of garlic. A fresh bulb contains 65% water, 7% nitrogen-containing and 26% nitrogen-free substances and 1.5% salts, including mineral salts, mucin, sugar, some fat, and 0.25-0.9% essential oils. H. Hager (21) mentions a smaller content (0.15%) of essential oils. The latter, according to Zörnig, appear as sulfur-containing compounds of the following formulas: Disulfide, $C_6H_{10}S_2$ - the carrier of the typical odor up to 60%, disulfide $C_6H_2S_2$ (allylpropyl sulfide), and further a substance of the formula $C_6H_{10}S_2$. - According to Semmler (16) there are contained in the essential garlic oil: 1) Disulfide $C_6H_2S_2$ (6%), 2) Disulfide $C_6H_{10}S_2$ (60%), 3) a substance of the formula $C6H_{10}S_3$ (20%) and 4) a substance of the formula $C_6H_{10}S_4$.

In the opinion of most researchers, garlic owes its effect precisely to these essential oils. Only Noether doubts that the effect of garlic is to be attributed to these organic sulfur compounds.

If we take into account the content of essential oils in garlic as indicated by Zönig and Hager, and also the fact that for the preparation of the tincture ones takes 40 parts garlic to 100 parts alcohol, the tincture contains 0.06 to 0.36% thereof, even assuming that all essential oils pass into the tincture.

General description of the methodology of the experiments

For the purpose of investigating the direct effect of garlic on the cardiovascular system, we made 86 tests on various isolated surviving organs - ear, kidney and heart of rabbit and heart of frog - by passage of a pure garlic solution and of such a solution together with another substance. By marking garlic 1:x in our experiments, we indicate thereby the dilution of the garlic tinc

ture with Ringer-Locke's solution. Since we used an alcohol extraction of the garlic, we sent through in all experiments without exception, before passage of the garlic, an alcohol solution + R.-L. solution in that concentration which corresponded to the content of the garlic solution to be tested, and in many tests we again sent through the same alcohol solution after the garlic. The effect of garlic was thus shown, as it were, against the background of the alcohol effect, and we evaluate the changes caused by the garlic by comparing the results upon passage of garlic and of alcohol. The same process was observed also upon introduction of additional stimuli (chloral hydrate, arecoline, atropine), which we ran through with alcohol before passage of the garlic.

I. Effect of garlic on the peripheral vessels

For this purpose, by the method proposed by N. P. Krawkow and described by S. A. Pissembskii (17), garlic solutions with R.-L. solution were passed through the vessels of an isolated rabbit ear. In 22 tests (on 8 ears) solutions of a concentration of 1:200,000 to 1:100 were used at approximately 20°C and a pressure of 70 cm water column. The numerical compilation of the test results will be found at the end of this paper (Table 1). All tests on the ear generally showed a weak and inconstant effect of garlic on the vessels. In two experiments (3 and 6) (concentration of the solutions 1:100,000 and 1:50,000) at first an increase to 3-4.4%) of the outflow of liquid was observable which lasted 8-12 minutes and was then followed by a reduction of the quantity of liquid (see Fig. 1 **).

Fig. 1. Experiment 3. o-o-o Garlic (
(1:100,000
(dots) Alcohol (

Fig. 2. Experiment 7. Garlic, alcohol, 1:25,000

Fig. 3. Experiment 15. Garlic, alcohol, 1:5,000.

In 13 tests with concentrations from 1:200,000 to 1:5000 there was observed at first a decrease of the outflow of liquid to 2 to 18%, and the constriction of the vessels persisted with slight fluctuations during the entire period of

passage of garlic (see tests 1, 2, 4, 5, 7, 9, 10, 11, 13, 14, 15, 20 and 21) (see fig. 2 and 3). In two experiments (12 and 22), the garlic (1:10,000 and 1:100) caused at first a constriction of the vessels (to 5%) lasting a few minutes and later changing to dilation to 18% (see fig. 4) in experiment 12, and a constriction for 2 minutes to 1.5% with subsequent dilation to 9% in experiment 22. In three experiments, vessel dilation was observed from beginning to end of the garlic passage, namely in experiment 18 (1:1000) to 3.8%, in experiment 8 (1:20,000) to 13.8% and in experiment 19 (1:1000) to 6.8% (see fig. 5). Lastly in 2 experiments (16 and 17) with concentrations 1:5000 and 1:1000 the garlic caused no variation of the vessel lumen at all (see fig. 6).

Fig. 4. Exp. 12. Garlic, alcohol, 1:10,000.

Fig. 5. Exp. 8. Garlic, alcohol, 1:20,000.

The cited experiments demonstrate the instability of the variation of the lumen of the peripheral vessels under the effect of garlic, which in some experiments caused vessel constriction, in others dilation, and in a third part a temporary constriction with subsequent dilation or vice versa, and lastly in some experiments no effect on the vessels at all. Moreover, a weak effect of the garlic on the ear vessels appeared even at highly concentrated solutions (1:500 and 1,100). An exception are experiments 4, 5, 10 and 14, where upon passage of alcohol and garlic solutions as well as during the following flushing with normal R.-L. solution a sharp, progressive decrease in the outflow of liquid was observed, which in these experiments is to be attributed, not to the effect of garlic, but to some change in the vessels themselves (embolism?).

Fig. 6. Experiment 17. Garlic, alcohol, 1:1000.

In all experiments the absence of any proportionality between the effectiveness of the garlic and the concentration of its solution passed through was evident.

It must be concluded from what has been said that garlic constitutes a weak

stimulus for the peripheral vessels.

II. Effect of garlic on kidney vessels

The passage of garlic through the vessels of an isolated rabbit kidney was carried out by the method of W. W. Sakussov (19). The kidney was thermostated at 40°C, the solutions were heated to 40°C and passed through under a pressure of 60 cm water column. For test results see Table 2.

In 3 experiments (23, 24, 32) a dilation of the kidney vessels to 2.7-7% lasting for a short time (2-10 minutes) with following constriction to 5.4-17% was perceptible upon passage of garlic (see fig. 7). In two cases the constriction of the kidney vessels was constant at 9.5% (exp. 28) and 47% (exp. 25). In the latter experiment, which with respect to the intensity of action is an exception, the progressive decrease in outflow of liquid even after subsequent flushing with normal R.-L. solution suggests some lasting alteration of the kidney vessels apparently independent of the garlic.

Fig. 7. Experiment 24. Garlic, alcohol, 1:40,000.

In 3 experiments (26, 27 and 29) only a vessel dilation to 3.6-9.5% was perceivable. In experiment 31, a slight vessel constriction (to 2.6%) was later followed by a considerable dilation (to 55%). In this experiment, a general instability was noticeable in the flow of the liquid with or without garlic. Great fluctuations in the vessel lumens are an exception and are not easily explained by the effect of garlic.

In all these experiments, the effect of garlic on the kidney vessels generally appears as weak as that on the ear vessels, as only in one case it attained a constriction of their lumen to 47% and in another a dilation to 55%, which must be attributed to some causes independent of the effect of garlic. Its effect was remarkable for the same instability as in the ear experiments. Thus, one and the same concentration of the garlic solution (1:1,000) caused in exp. 29 a vessel dilation to 6.5%, in exp. 30 a constriction with subsequent dilation to 7%, and in 31 a dilation to 55%.

On the basis of our experiments, no predominant dilating or constricting

action of garlic on the kidney vessels canbe seen. Nor is there an increase of the garlic effect with increasing degree of concentration of the solutions passed through.

III. Effect of garlic on the coronary vessels of the heart

With the passage of garlic solution through the coronary vessels of a rabbit heart we made 10 experiments, including 6 (33-38) on hearts stopped by Krawkov's method (18) by preliminary passage of a strophanthin solution (Strophanthin Merk) 1:50,000. After its stoppage, flushing with normal R.-L. solution was carried out for 50 minutes. In 4 experiments (39-42) the state of the lumen of the coronary vessels was studied on three active isolated hearts. The amount of liquid flowing through was measured in experiments 33-41 by totaling the number of drops per minute, and in experiment 42, the flowing liquid was collected in a graduated cylinder and noted in cubic centimeters. The results obtained are shown in Table 3.

In the passage of garlic solution through the vessels of a heart stopped by strophanthin there was observable either the complete absence of a modification of the vessel lumen at the solution concentrations 1:1000 (exp. 36), 1:500 (exp. 37) and 1:100 (exp. 38) (see fig. 8) - or a certain vessel constriction at the concentrations 1:20,000 to 21.5% (33), 1:5000 to 15% (34) and 1:1000 to 12.5% (35).

We obtained entirely different results when passing the garlic solution through the vessels of the working heart. In experiments 40, 41 and 42, the number of drops increased greatly during the second and third minute upon passage of the garlic solution 1:500 and continued to increase, reaching in experiment 42 a frequency such that counting became impossible (more than 180 drops per minute) (see fig. 9). This state of the vessels persisted during the entire experiment for 20 minutes. In exp. 39, with a 10 times weaker garlic solution 1:5000, the quantity of the flowing liquid was increased from 9.5 cc to 12 cc (+ 26.3%), and except for a slight deviation in the 6th minute the vessels remained in such a state during the entire time of passage of garlic solution (more than 18 minutes) *see fig. 10). Thus, from the exper-

iments on passage of garlic solution through the coronary vessels of an isolated active rabbit heart, the increase in the passage capacity of the heart vessels became clear (in exp. 42 more than 2½ times). As this passage capacity of the vessels depends not only on a spontaneous variation of the lumen of the coronary vessels, but also on the tonus of the myocardium, it was of interest to record also the heart activity. This was done in three cases. In experiment 39 it was found that simultaneously with the increase of liquid passage through the coronary vessels to 21-26.3% a decrease of the amplitude of the heart contraction initially to 22% and at the end of garlic passage to 55% was observable. This decrease in amplitude took place at the expense of the reduction of the systole at diastolic heart weakening. It should be noted here that according to the degree of amplitude reduction and increase of the diastolic heart weakening, no increase in liquid flow took place. The amount of liquid flowing through was uniformly increased almost the entire time, while the amplitude decreased progressively and the diastolic heart weakening increased. It follows that the passage capacity of the heart vessels increased not only at the expense of their passive dilation (due to weakening of the myocardium tonus), but, if only in part, at the expense also of the active dilation of the coronary vessels. In two other experiments, where simultaneously the cardiac activity was recorded and the outflowing drops were counted, it was again not possible to detect a proportionality between the degree of decrease of the contraction amplitude, the diastolic heart weakening, and the increase in passage capacity of the heart vessels. Such an effect of garlic must be stressed and in it must be seen an explanation of its at least partially favorable effect in many cases of sclerosis of the heart that Pouillard, Schlesinger, Vincent, Suncori, S. May, Schwahn and others have reported. The absence of the vessel-dilating effect of garlic on a heart stopped by strophanthin may possibly be explained in the sense that under the influence of the strophanthin those elements which in the vessels are stimulated by the garlic have lost their reactivity.

Fig. 9. Experiment 42. Garlic, alcohol, 1:500.

Fig. 10. Experiment 39. R-L solution pure, garlic (cross-hatched), alcohol, 1:5000.

IV. Effect of garlic on the isolated heart

For the study of the direct effect of garlic on this organ we made 44 experiments, including 20 on 10 rabbit hearts and 24 on frog hearts.

Isolation of the rabbit heart was performed by the usual method of Langendorff, and of the frog heart according to Beresin (20) and the heart activity by means of the kymograph. To clear up some details of the effect of garlic, its solution was passed through rabbit and frog hearts weakened by means of preliminary experiments in the apparatus over 2-4-6 hours and through the frog heart alone, poisoned by means of chloral hydrate. To clear up the mechanism of action of garlic, experiments were made with preliminary and simultaneous passage of atropine and arecoline solutions plus garlic, so as to act on the N. vagus as the supplementary agent.

A. Experiments on the rabbit heart

We carried out 17 experiments with passage of the garlic solution. The concentration of the solutions varied between 1:200,000 and 1:500. All disclosed a distinct effect of the garlic on the heart activity. The intensity and duration of the effect varied with a number of conditions - solution concentration, individual fluctuations of the reaction of the heart, its weakening by the preceding experiment in the apparatus, etc. The results of these experiments are compiled in Table 4. Thus, in exp. 43 (see Table 4 and Fig. 11) the garlic solution 1:200,000 passed after 26 minutes of continuous passage of alcohol, increased the number of contractions in the second minute to 12%, and at the end of the same minute the amplitude decreased from 74 mm to 67 mm (-9.4%). In the course of the third minute, the amplitude dropped to 17 mm (-23%), and the rhythm was accelerated to 136 (+39%). In the 4th minute, the amplitude rose in comparison with that during the preceding minute to 1.5 mm,

and the rhythm decelerated greatly (to 96). At the same time, the arrhythmia took on in the 3rd and 4th minute the character of a sinus arrhythmia. In the 5th minute the amplitude decreased to 55 mm (-25.7%) and the rhythm decelerated to 82 (-16%). This reduction of the amplitude during the entire experiment occurred at the expense of the reduction of the systole and of the diastolic heart weakening. Approximately the same cardiac activity persisted with insignificant fluctuations during passage of garlic for 30 minutes. At the end of this time the amplitude was 24.3% lower than the original, and the number of contractions was 1% fewer than the original. Despite 1-hour flushing with the normal R.-L. solution, the amplitude continued to decrease gradually. In this experiment, therefore, the garlic showed, regardless of the low concentration of its solution, a lasting and constant action on the amplitude of the heart contractions in the sense of their reduction and brought about a change of rhythm which manifested itself during the first minute by a significant acceleration (maximum 39%), later by a visible retardation (maximum 18%), and between the 20th and 30th minute by the return to the original frequency. This experiment appears to be typical.

Fig. 11. Experiment 43 (half natural size).

Minute of passage of the solutions. Alcohol 1:200,000. Garlic 1:200,000.

Let us briefly cite the results of other experiments we made. At higher concentrations (1:100,000 and 1:50,000) we observed in most cases (exp. 44, 45 and 47) a reduction of the amplitude to 37 - 35 - 72%, the heart soon standing still completely inthe last experiment. In this same experiment, where the heart had been fatigued in the apparatus by continuous effort and had been poisoned by previous passage of a garlic solution 1:200,000, the garlic showed a highly inhibiting effect. The rhythm did not change as consistantly in these 3 experiments; in the first (44) it was slowed to 12.5%, in the second (47) it was sometimes slow and sometimes fast, and in the third (45) it was continuously accelerated. In still another experiment (46) the same concentration of garlic solution caused an insignificant increase of amplitude (to 8%) and had

little effect on the rhythm. The passage of still higher concentrations, 1:25,000 (exp. 48 & 49), 1:6250 (51), 1:5,000 (52 and 53), 1:1000 (54 & 55), 1:500 (56 & 57), caused reduction of the amplitude varying between 17 and 98%, and led to complete stoppage of the heart in experiment 48. An exception are the results obtained in experiment 46, where the garlic produced a slight increase of the amplitude. In all our experiments, excepting 46, the inhibiting effect of garlic on the isolated heart is clearly seen, reflected in a reduction of the amplitude. Such an effect was observed nearly always; it was more intensive and lasted longer than the opposite effect of heart stimulation. In some experiments the inhibition of the heart by the garlic led to its stoppage (47 & 48).

The effect of garlic on the rhythm of the heart contractions was less constant and weaker than that on the amplitude. In some of the experiments the rhythm slowed down right from the start, and this deceleration continued throughout the passage of the garlic solution (exp. 44, 50, 51, 53). In other cases the garlic induced a temporary acceleration of the rhythm, lasting 2-5 minutes, followed by deceleration (exp. 43, 52, 55, 56). In two experiments (46, 49) a slight deceleration of the rhythm was seen in the first minute of passage of garlic solution, changing to acceleration in the 4th to 5th minute. In the last part of our experiments finally the rhythm of the contractions remained accelerated from beginning to end (47, 54, 57). The changes in rhythm under the influence of garlic were not very pronounced and in most cases did not exceed 4-8% and only in 5 experiments they alternated with 17-39%. Generally retardation of the rhythm predominated. Besides, the absence of a parallelism or proportionality between the amplitude variations occurring under the influence of garlic and the rhythm of the contractions was noted in our experiments. In some of our experiments a significant decrease in amplitude was accompanied by insignificant variations of the rhythm (50-54). Besides, the decrease of amplitude was not always accompanied by deceleration of the rhythm; sometimes it was even seen to be accelerated (45, 49, 57).

A regular increase in the intensity of the garlic effect at increased con-

centrations of solution was not observable. On the contrary, in some cases more concentrated solutions had a weaker effect than several times weaker solutions (cf. the results of exp. 43, 44, 46, 47, 49 and others). In all our experiments it is striking how quickly the action of garlic on the heart manifests itself. In most cases definite changes in cardiac activity attributable to the garlic could be seen in the second, at the latest the third, minute. In some experiments (e.g. 54) the garlic effect appears already in the first minute of passage of the solution. As to the washing out of this poison, this always occurred very slowly, and it was possible to do so to the full extent only in a small portion of the tests by passage of garlic solutions of weak concentration; but in most cases the garlic effect was prolonged, was little reversible by subsequent passage of normal R.-L. solution,and could be eliminated only in part. The reson for this seems to be that the action of the garlic is due to the essential oils contained in it, which dissolve in the lipoids of the tissues and are little soluble in aqueous solutions and hence difficult to flush out with them. Habituation to garlic was not observable in our experiments made on isolated vessels and on the heart.

B. Effect of garlic on frog heart

We made 32 experiments on the isolated frog heart (see Tables 5, 6 & 7). In the first part thereof we examined the action of garlic alone, for which we passed solutions of from 1:10,000 to 1:100. These experiments are compiled in Table 5. At a concentration 1:10,000 (58) the garlic resulted in a slight increase of the amplitude (+5.5%) and a deceleration of the rhythm (7.5-23%), and at a concentration 1:5,000 (59) initially in a reduction of the amplitude to 25-37%; later the amplitude increased somewhat and stayed 12-13% below the original. Both experiments showed that the frog heart is less sensitive to garlic than the rabbit heart. To obtain a clear effect, we therefore used in the further tests more concentrated garlic solutions, which resulted in a reduction of the amplitude and a deceleration of the thythm (see 59, 60, 62). Different results were obtained in exp. 61, where under the influence of garlic

an increase of the amplitude to 3-11% at deceleration of the rhythm to 16% occurred, and in exp. 63, where the garlic in a concentration 1:200 brought about in the first 3 minutes a significant reduction of the amplitude (-71, -43%) and irregular contractions, observed in groups of 2-3 contractions with pauses of 14-32 seconds. In the 14th to 15th minute the rhythm of the contracting became regular and accelerated (+28%) and the amplitudes became more regular and stayed 21-28% above the initial level (see fig. 12). At concentration 1:100 the garlic caused stoppages of the heart for 80 seconds already in the first minute (exp. 64); this repeated between the 13th and the 15th minute and lasted for 3 minutes; the stoppages repeated several more times, lasting 15-30 seconds. In general the experiments with the frog heart showed a less constant and less strong action of the garlic than with the rabbit heart, producing sometimes a reduction sometimes an increase of the amplitude, and sometimes a deceleration sometimes an acceleration of the rhythm. When passing more concentrated solutions, we found a retardation of the contractions, the occurrence of longer pauses between them and even temporary stoppage of the heart. In the subsequent experiments with frog hearts we tried to clarify the mechanism of the garlic effect, for which purpose we passed through together with it vagotropic substances - arecoline and atropine.

Fig. 12. Experiment 63 (natural size).

Minute of passage of the solutions. Alcohol 1:200. Garlic 1:200.

V. Joint action of garlic and arecoline on frog heart

In 11 experiments (67-77) we investigated simultaneously arecoline solutions (Arecolinum hydrobrom. Merck) in dilutions of 1:200 million to 1:10 million and garlic of 1:2000 to 1:100. The numerical results appear in Table 6. In experiments 65 and 67, arecoline solution 1:10 million caused in the diastole a complete stoppage of the heart, which in the first experiment disappeared after flushing with normal R.-L. solution for 16 minutes, and in the second immediately after applying a drop of atropine solution (1:1000). Thereafter we used weaker arecoline solutions.

Let us briefly report the results. In all these experiments, solutions of arecoline + alcohol were passed through for 20-30 minutes, and after establishmentof a certain stability of the cardiac activity, these solutions were replaced by solutions of arecoline + garlic in the same concentrations and the cardiac activity was compared with that caused by the arecoline-alcohol solution. Always without exception a reduction of the amplitude was observable under the influence of garlic; in some of the experiments (68, 73, 75, 76) this attained a value smaller by 50-75% than that found when the arecoline-alcohol solution was passed through. The number of contractions was always fewer, and in a number of cases a temporary heart stoppage lasting 10 seconds to 2 and 3 minutes or even longer occurred during passage of garlic + arecoline. Noteworthy are the results of experiment 76, where arecoline 1:50 million with alcohol 1:200 caused a reduction of the amplitude to 30%, a slowing of the rhythm to 33%, and arecoline-garlic solutions of the same concentrations, passed through 30 minutes later, showed a still greater reduction of the amplitude (to 55%), a deceleration of the rhythm (to 62%) and even temporary stoppage lasting 10 seconds in the third minute. Upon subsequent passage of the same arecoline-alcohol solutions, the amplitude increased from 4 to 8 mm (less by 20% than at the beginning of this experiment and more than by 100% upon passage of arecoline + garlic) and the number of contractions increased from 9 to 30 (more than by 233% of that which occurred on passage of garlic, see fig. 13).

The result of the repeated passage of alcohol + arecoline after passage of garlic + arecoline therefore leaves no doubt that the maximal reduction of the amplitude and deceleration of the rhythm was caused in this experiment by the garlic. In the experiments cited in the preceding chapter with pure garlic solutions we had observed a reduction of the amplitude and deceleration of the rhythm, but never did the changes reach so high a degree as with the passage of garlic + arecoline. On the other hand, a weak pure arecoline solution did not produce so strong an action as with the simultaneous passage with garlic (except in the two experiments with solutions of concentration 1:10 million).

When we added garlic to the arecoline, as was done in all our experiments, this supplementary effect of the garlic led - after preliminary passage of pure arecoline and after establishment of a certain stability of the cardiac activity - to a further, still greater reduction of the amplitude and deceleration of the rhythm, and in a number of experiments to complete stoppage, as was obtained by us under the influence of pure arecoline when using a 10-20 times stronger solution thereof. Besides, in exp. 76, the great decelerations of the rhythm and reductions of the amplitude occurring under the influence of arecoline + garlic were considerably reduced by the subsequent passage of arecoline + alcohol.

Fig. 13. Experiment 76 (natural size).

Minute of passage of the solutions

R.-L. solution, pure - Arecoline 1:50 million and alcohol 1:200 - Arecoline 1:50 million and garlic 1:200 - Arecoline 1:50 million and alcohol 1:200

All these experiments permit the assumption that one of the points of attack of the action of garlic seems to be the endings of N. vagi and that garlic stimulates them in that, due to a slowing of the rhythm, it entails a decrease of amplitude and, in the cases of strong action, stoppage of the heart. The action of the garlic on the heart is particularly strong at increased tonus (in our cases by arecoline) of the peripheral endings of the vagus.

VI. Joint action of garlic and atropine on the isolated heart

On the assumption of the action of garlic on the vagus, we made experiments with previous and simultaneous passage of atropine solution (Atropinum sulfur. Merck) and garlic in concentrations 1:1 million to 1:100,000 on the rabbit (78-82) and frog heart (83-86) - see Table 7 - with the intention to paralyze the vagus and to examine the effect of garlic under such conditions. These experiments were carried out as follows: Solutions of atropine + alcohol were passed for 25-30 minutes, and then, as soon as the atropine had shown its usual action on the vagus, solutions of atropine + garlic of the same concentrations were incorporated.

Fig. 14. Experiment 83 (natural size).

Minute of passage of the solutions

R.-L. solution, pure - Atropine 1:100,000 and alcohol 1:200 - Atropine 1:100,000 and garlic 1:200.

Our experiments with atropine + garlic on the rabbit heart gave no specific results, and regardless of the atropinization a certain inhibiting effect was detectable under the influence of garlic. It may be concluded from this that in warm-blooded animals garlic exerts, in addition to the action by way of the vagus, apparently also a direct inhibiting action on the actual heart apparatus.

Other and more definite results were obtained in our experiments on frog hearts. In all experiments with simultaneous passage of garlic + atropine we saw no deceleration of the rhythm of the heart contractions. This serves as direct proof that this consistent garlic effect is caused by the stimulation of the N. vagus, and since in these experiments the vagus was paralyzed by atropine, the garlic therefore produced no deceleration of the rhythm.

Thus these experiments on the heart confirm the above-mentioned assumption of the stimulating effect of garlic on the N. vagus, which Noether and Bachem had reported on the basis of the analysis of clinical phenomena caused by garlic in the digestive apparatus.

Besides this proof of the vagus effect of garlic, these experiments disclosed another, no less interesting fact, namely the simultaneous action of garlic on the accelerating apparatus of the heart.

Thus in exp. 83 with the passage of atropine + garlic no change in amplitude or rhythm was detectable in the first minute, but in the 10th minute the amplitude increased from 9.5 mm to 20 mm (+110%), in the 24th minute still more (+121%), later it decreased only little (to 110%) and stayed there to the end for 39 minutes. The number of contractions also increased in the 10th minute by 10% and at the end of the experiment by 13.5% (see fig. 14).

In the other experiments the effect of garlic + atropine was analogous, but

was not as pronounced. Thus for example in exp. 84 the amplitude increased to 7%, the number of contractions to 14%; in exp. 85, the former to 17.7% and the latter to 18%. In exp. 86, garlic 1:100and atropine 1:100,000 led to increase of the amplitude (20%) and of the number of contractions (23%). The repeated passage of atropine + alcohol after atropine + garlic in experiments 85 and 86 reduced the amplitude to 17% and 23%, respectively, and the number of contractions to 4% and 11.5%, respectively. Similar results of the effect of garlic on the atropinized frog heart convincingly suggest that garlic manifests its effect on the frog heart also when the vagus is paralyzed by atropine, but the action is a different one from that which we had seen in the earlier experiments without atropine, in that it appears in the increase of the amplitude and acceleration of the rhythm. It may be concluded from this that garlic stimulates also the acceleration apparatus of the heart, but that this effect is very much weaker than the stimulating effect on the vagus nerve, so that, when the vagus is intact, it is usually either completely suppressed by its phenomena of stimulation (in the plurality of cases) or it is weak and of short duration.

Conclusions

The results we obtained in the experimental study of the action of garlic on the heart and vascular system are of indubitable interest, both in the sense that they clarify some details of this action and that they manifest the mechanism of action. In the total organism, of course, the action of each stimulus and its results are much more complicated than in an experiment of short duration on the isolated organ, and it must be borne in mind that also the action of garlic in the total organism may differ, quantitatively as well as qualitatively, from what we saw in our experiments. Besides, in appraising the action of garlic on the circulatory apparatus, also its effect on the digestive apparatus must be taken into account, because of the possibility of an indirect influence on the circulation - as discussed in publications of Tilger, Bonem, Bachem, Noether, Roos, Plant and others. It should be noted here that our investigations permit the conclusion that the lowering of the

blood pressure obtained by numerous authors after the use of garlic, in patients as well as in the animal experiment, is brought about by its direct - or partially direct - action on the heart, probably by stimulation of the inhibiting cardiac apparatus. This the clinician must take into consideration when using garlic as a medicament, and he must weight in the individual case whether a lowering of the blood pressure by this means appears desirable for the patient.

The results of our studies permit us to draw the following general conclusions:

1. Garlic causes a slight and inconstant alteration of the lumen of the peripheral vessels (of the ear) and of the inner organs (kidney).

2. The passage capacity of the coronary vessels of the heart (of the rabbit) increases significantly under the influence of garlic, this being dependent on their active dilation and the weakening of the myocardium tonus.

3. Garlic has an inhibiting effect on the rabbit and frog heart, in that it reduces the amplitude of the contractions by decrease of the systole and weakening of the myocardium tonus and decelerates the rhythm of the contractions.

4. Garlic manifests its action in the stimulation of the endings of the N. vagus and in part by direct action on the myocardium. As is evident from the experiments with atropinized frog hearts, garlic has also an action stimulating the N. accelerans cordis, although it is weak and inconstant and it is usually suppressed, in case of intact N. vagus, by stimulation phenomena thereof.

5. The action of garlic on one and the same organ varies within wide limits and not always proportionally to the concentrations of its solutions, and it depends on individual fluctuations of the reaction of the organs to it.

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(**) On the ordinate, the quantity of solution flowing in one minute is stated in number of drops; the spacing of the horizontal lines in figures 1-8 corresponds to 5 drops, in fig. 9, 20 drops. On the abscissa, the time of passage of the liquid is shown in minutes. Other conditions are indicated specially at each curve.

Table 1. Effect of garlic on the vessels of the isolated ear.

Test No.	Concentration of the garlic solns.	Passage time of the solutions in minutes				
		1-5	6-10	11-20	21-30	31-60
		Variations of the vessel lumens in %				
1	1:200,000	-2.5	-3.7	-5	- 8.7	-5; -4
2	1:100,000	-3	-6	-9	-12	
3	1:100,000	+2;+4.4	+2	+2.0	-2;-5.5	
4	1:100,000	-6	-24	-36	-54	
5	1:50,000	-5	-10	-15	-25	-40
6	1:50,000	+3	+1.5; 0	- 1.5		
7	1:25,000	-5;-6.5	-8; -10	-10	-6.5; -5	- 5
8	1:20,000	+10.3	+12	+13.8		
9	1:20,000	-3	-2	-4	-3	-4
10	1:20,000	-8.3	-25	-50		
11	1:20,000	0	0	- 2	- 1.2	
12	1:10,000	-5	+3.3; 0	+ 6.5	+ 14.8	+ 18
13	1:10,000	-2	-2	- 8.8	- 10.5	- 13
14	1:10,000	0	0	-20		
15	1:5,000	-10.7	-16.6	-18	- 3.6	
16	1:5,000	0	0	0		
17	1:1000	0	0	0		
18	1:1000	+2	+ 3.8	+2.5	+ 3.8	
19	1:1000	+5	+ 5	+6.8		
20	1:1000	-2.6	-1.3	-2.6	- 1.3	
21	1:500	-2	-2.9	-4.4		
22	1:100	-1.5; +3	+6	+8	+ 7.7	+ 6

Table 2. Effect of garlic on the vessels of the isolated kidney.

Test No.	Concentration of the garlic solns	Passage time of the solutions in minutes				
		1-5	6-10	11-20	21-30	31-60
		Variations of the vessel lumens in %				
23	1:200,000	+4; -4	-11	-15;-11		
24	1:40,000	+2.7	+ 1.3	-1.3	-2.7	-5.4
25	1:20,000	-13	-16.5	-19	-33	-47
26	1:20,000	0	0	+5	0;+9.5	
27	1:10,000	0	+ 3.6; 0	+3.6		
28	1:5000	-5	-9.5	-5	-9.5	
29	1:1000	+3	+6.5	+3	+ 6.5	
30	1:1000	0	-7	0	+ 7	
31	1:1000	-2.6	+20	+31	+55	
32	1:500	+5	-10	-17		

Table 3. Effect of garlic on the coronary vessels of the heart.

Test No.		Conc. of garlic solns.	Passage time of the solutions in minutes					
			1-3	4-6	7-9	10-15	16-20	21-25
			Variations of the vessel lumens in %					
33	Heart stopped	1:20,000	-14.3	-14.3	-21.5	-21.5	-21.5	
34	by strophan-	1:5000	- 7.7	-11.5	-11.5	-11.5	-15	
35	thin	1:1000	0	-12.5	-12.5	-12.5	0	
36		1:1000	0	0	0	0		
37		1:500	0	0	0	0		
38		1:100	0	0	0	0		
39	Functioning	1:5000	+26.3	+ 21.5	+26.3	+26.3	+26.3	
40	heart	1:500	+ 3	+40	+57	+96		
41		1:500	+ 7	+29	+55	+80	+> 90	
42		1:500	+ 6	+60	+152	+>180	+>;80	+>;80

Table 4. Effect of garlic on isolated heart of rabbit.

Test No.	Conc. of garlic soln.	Variations of the cardiac activity in % in the 3rd		5th		7th		10th		15th		20th		25th		Minute of passage
43	1:200,000	-23	+39	-25.7	-16	-21.6	-18	-19	-15	-19	- 8	-16	-3	-24.3	-1	
44	1:100,000	-12.5	-8	-37	-12	-16.6	-11	-12	- 8	-12.5	- 4.3	-12.5	-4.3			
45	1:100,000	- 5	0	-25	+8.2	-30	+14	-25	+10	-17.5	+ 7					
46	1:50,000	+5.5	-2	-5.5	+1	+4	0	+5.5	+2	+ 8	+4.4	+1.4	+ 7			
47	1:50,000	-50	-30	-56	+34	-40	- 7	0	+7	-56	+10	-72	Stoppage of heart			
48	1:25,000	Complete stoppage of heart														
49	1:25,000	-2	-2	-17.3	+ 2	-11.5	0	-11.5	+ 1	- 14	0	- 16	-2			
50	1:20,000	-19	-1	-30	- 5.5	-36	-6.6	-13	-7.7	- 16	-10	-18	-7			
51	1:6250	-28	-2	-29	- 2	-33	- 3	-43	-4	- 36	- 6					
52	1:5000	-22	+1.7	-22	- 8	-22	- 2	-30	-3	-41	- 4	-56	-2.5			
53	1:5000	-12	-0.9	-24	-0.9	-32	-18	-24	-3.6	-21	- 6	-16	-7			
54	1:1000	-33	+2.9	-48	+4	-65	+ 7	-98	+8.7							
55	1:1000	-19	+8	-37	+18	-19	- 9	0	-8	0	-10	-12.5	-10	-30	-12	
56	1:500	-11	0	-53	+5.7	-44	- 2	-33	-6.6	-55	-10	-59	-29	-66	-36	
57	1:500	-12	+3	-25	+11	-19	+12	-12.5	+14	-12	+17					

(a = Amplitude; b = Number of contractions)

Table 5. Effect of garlic on the isolated frog heart.

Test No.	Conc. of garlic soln.	Variations of the activity in % in the Minute of passag													
		3rd		5th		7th		10th		15th		20th		25th	
58	1:10,000	+5.5	-7.5	+ 5.5	- 7	+5.5	- 8	+ 5.5	- 13	+5.5	-17	+5.5	-23		
59	1:5000	-25	-3.2	-32	-3.2	-37	-3.2	-23	-3.2	-18	-3.2	-13	-3.2	-12	-3.2
60	1:2000	- 7	-9	-10	-9	-14	-10	-10	-4,5	-14	-4.5	-18	-4.2		
61	1:1000	0	-8	+ 3	-10	+ 5	- 9	+11	-12	+11	-16	+11	-16		
62	1:1000	+4	0	- 4	- 7	- 4	-13	- 6	-13	- 7	- 9	- 8	-13		
63	1:200	-71	-21.4	-36	-32	-43	-50	- 7	-12	+21	+28	+28	+28		
64	1:100	1-2 minutes stoppage for 80 sec.		-46	+63	-30	+14	-32	+ 4	13-15 minutes Stoppage for 3 minutes					

Table 6. Total action of garlic and arecoline on isolated frog heart.

Test	Solutions and their concentrations	Variations of the cardiac activity in % in the minute of passage													
		3rd		5th		7th		10th		15th		20th		25th	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b
65	Arecolin 1:10,000,000	Sudden complete stoppage of heart, disappears after flushing for 16 minutres													
66	Arecolin 1:10,000,000	Same, disappears after applying drop of stropine solution													
67	Arecolin 1:200,000,000 Garlic 1:2000	0	- 3	-25	- 9	-30	-13	Complete stoppage of heart, disappears after applying drop of stropine solution							
68	Arecolin 1:200,000,000	- 8	-12	-30	-16.5	-50	-16.5	Same stoppage							
69	Arecolin 1:100,000,000	16	0	-33	-14	Complete stoppage for 2½ minutes									
70	Arecolin 1:100,000,000 Garlic 1:1000	- 2	-4.3	-14	- 7	-18	-14	-21	-20	-21	-20	-14	-11	- 3	-4.3
71	Arecolin 1:100,000,000 Garlic 1:500	-4.3	-4.3	-13	- 4	-19	- 9	-26	-13	-13	- 4	- 6	0	- 1	0
72	Arecolin 1:50,000,000 Garlick 1:500	Stoppage for 45 seconds		-14	-41	-19	-30	-21.8	-29	-16	-11	-12	- 8		
73	Arecolin 1:100,000,000 Garlic 1:200	-75	-14	Stoppage for 2 minutes		-62.5	-10	-46.6	- 8	-46	- 8	-40	- 6		
74	Arecolin 1:100,000,000 Garlic 1:200	-15.4	-13	-23	-20	-39	-25	-36	-25	-36	-25	-23	-22		
75	Arecolin 1:50,000,000 Garlic 1:200	-23	-12	-29	-18	-33	-24	-50	-24	-54	-20	-50	-16	-50	-14
76	Arecolin 1:50,000,000 Garlic 1:200	- 7	-11	-27	-36	-31	-40	-36	-44	-42	-50	-40	-56	-55	-62
		and stoppage for 10 sec.													
77	Arecolin 1:100,000,000 Garlic 1:100	- 8	-33	-12.5	-44	-25	-24	-25	-10	-20	0	-14	-5		

Table 7. Total action of garlic and atropine on isolated heart.

Test No.	Solutions and their concentrations	Variations of the cardiac activity in % in the minute of passage													
		3rd		5th		7th		10th		15th		20th		25th	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b
							Rabbit heart								
78	Atropine 1:500,000 Garlic 1:50,000	- 5	0	-20	-1.5	-25	+ 1.5	-15	+ 3	-14	+ 4.5				
79	Atropine 1:250,000 Garlic 1:25,000	-26	+ 1	-46	+ 3	-51	- 4	-61	-14	-61	-25	-61	-36		
80	Atropine 1:100,000 Garlic 1:50,000	+ 3	+ 1	-14	+ 4	- 5	- 2	-17	-18	-34	-35	-43	-33		
81	Atropine 1:100,000 Garlic 1:50,000	-20	+ 5	-20	- 8	-20	+10	-20	-12	-21	+14				
82	Atropine 1:100,000 Garlick 1:5000	- 5	- 1	-8.7	-1.5	-29	- 2	-40	- 5	-54	- 9	-70	-12		
							Frog heart								
83	Atropine 1:100,000 Garlic 1:200	0	0	+ 4	0	+10	0	+110	+10	+110	+10	+116	+12	±121	+13.5
84	Atropine 1:100,000 Garlic 1:200	0	+5	+4.5	+5	+ 5	+14	+ 7	+14	+ 7	+14	+ 7	+14		
85	Atropine 1:100,000 Garlic 1:200	-2	0	- 8	0	+13	+ 9	+17.7	+11	+17.7	+16	+16	+18		
86	Atropine 1:100,000	+3	-4	-10	0	+15	+ 2	+18	+21	+20	+22	+20	+23	+15	+18

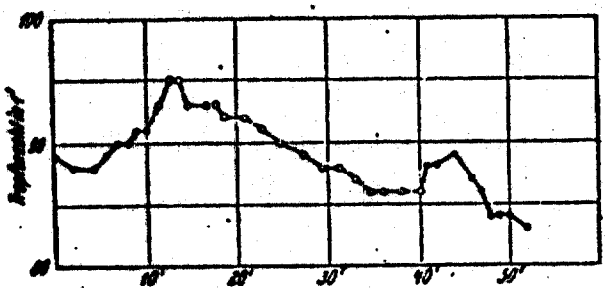


Fig. 1

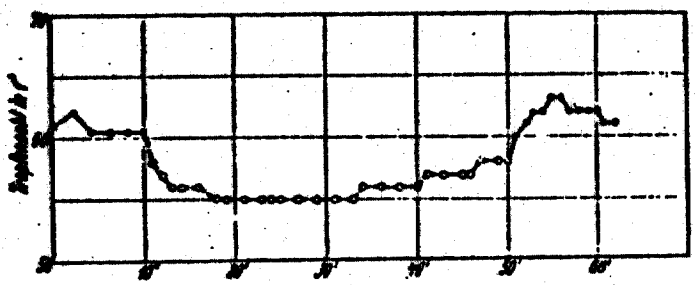


Fig. 2

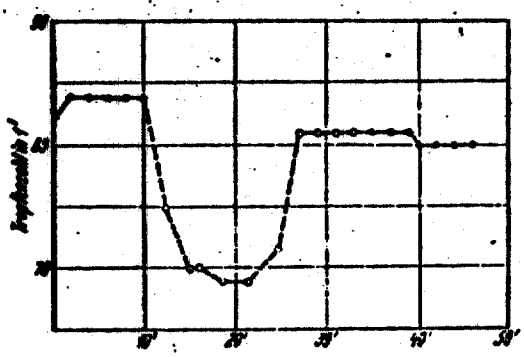


Fig. 3

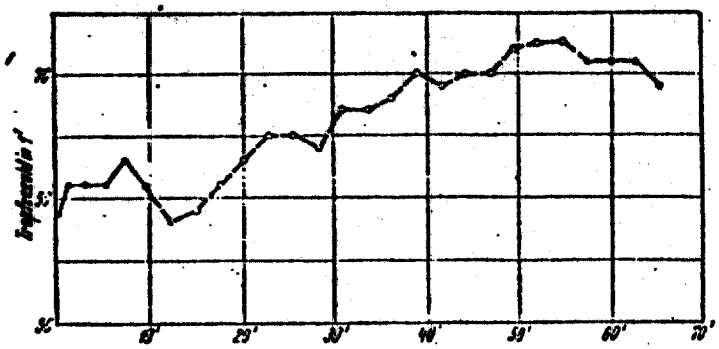


Fig. 4

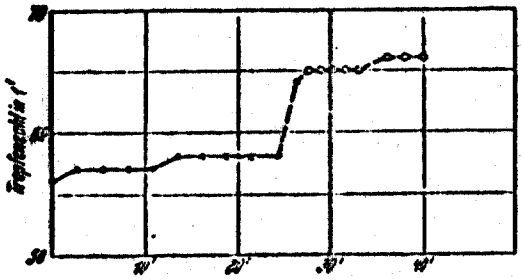


Fig. 5

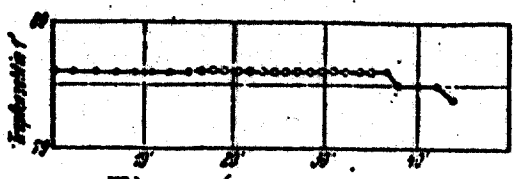
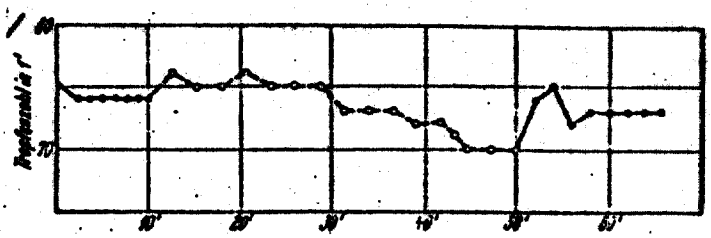


Fig. 6



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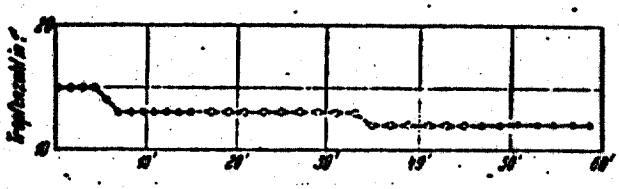
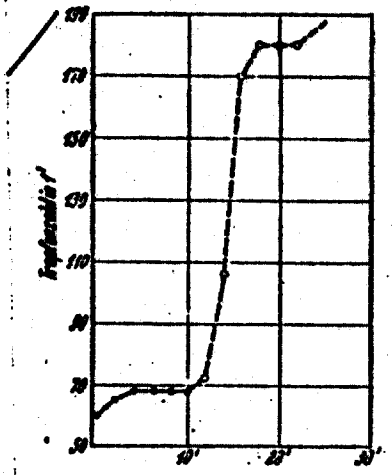


Fig. 8



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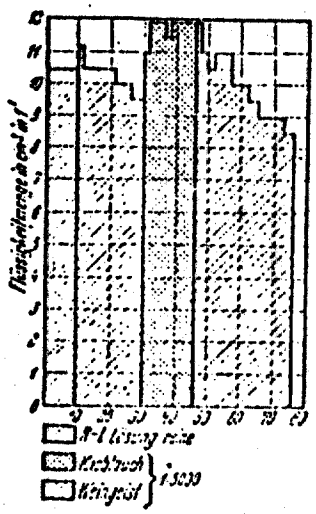
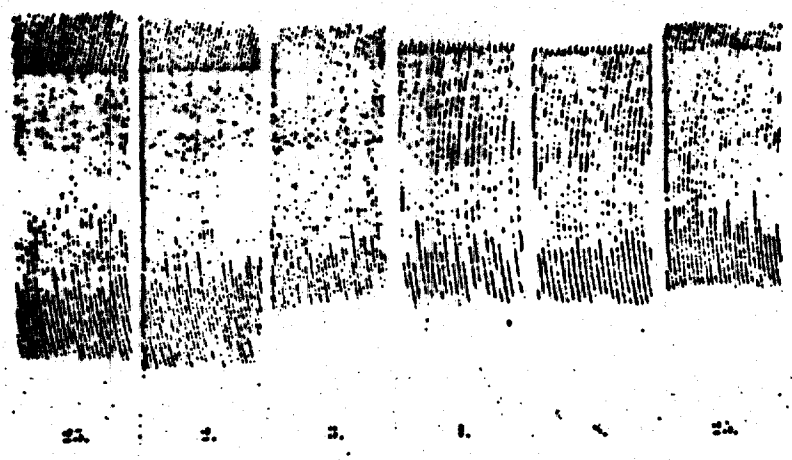


Fig. 10



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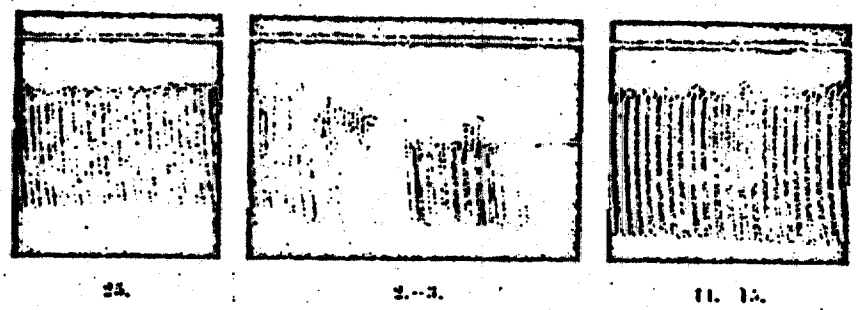

Fig. 12

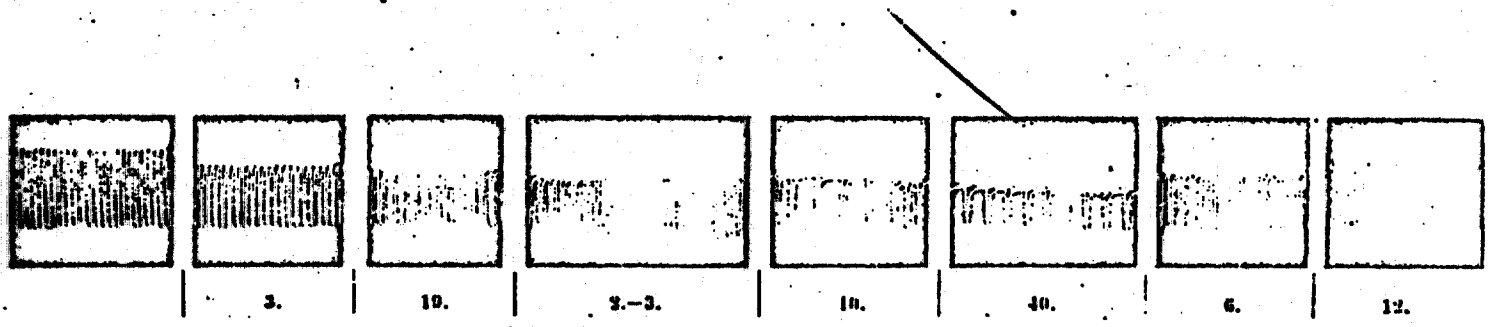

Fig. 13

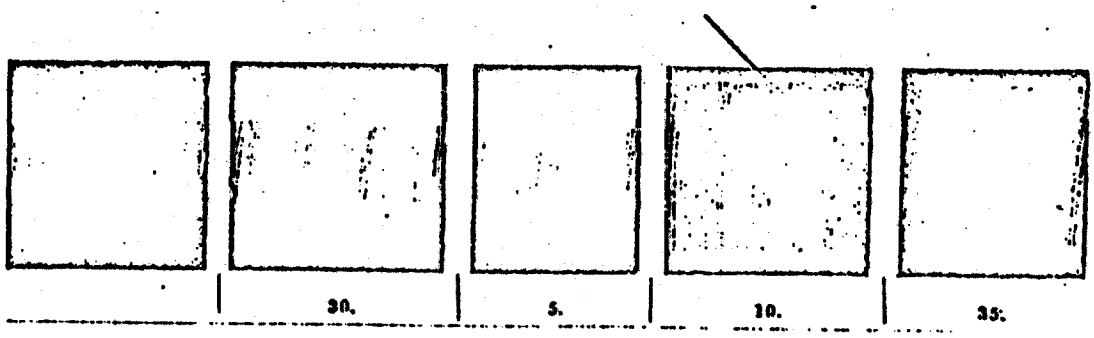

Fig. 14

(Italian, pp. 282-3)

Calcemia and the chemical composition of the bones during prolonged administration of garlic juice

by Giuseppe Sanfilippo

(Institute of Pharmacology, University of Catania)

Catania Section - Session of April 1, 1946

By earlier research (1) I have shown that garlic juice, given over a long period of time, causes increased calcemia in the dog.

I thought it would therefore be interesting to extend the experiments to another animal species. The tests were carried out on 9 male guinea pigs of nearly equal body weight (about 400 g), fed with constant daily quantities of bran and green fodder. Three were kept as controls; the others were subjected to the action of garlic juice, obtained by pressing. It was administered by mouth daily at the dose of 1 cc perkg of body weight, mixed with little bran, just before giving the morning food ration. The animals thus treated always appeared normal and gained weight considerably. As I had found in the experiments on dogs that the highest values of the calcemia were reached between the 14th and 28th day of the experiment and that they then became normal again, although the test conditions remained unchanged for another month, I determined the calcemia in 3 of these guinea pigs after 3 weeks and in 3 after 2 months of daily administration of garlic juice. The animals were sacrificed by bleeding; a glass cannula was introduced into the carotid and the blood was collected directly in a centrifige test tube. In this way hemolysis was strictly avoided which, even if it is of minimal degree, notably alters the values of the analytical results. By the method of Kramer and Tisdall I analyzed the calcium in 2 cc of serum. After the bleeding, I disarticulated the teeth and the femurs and analyzed the calcium in the ash (precipitation as oxalate and then in acid medium titration with a 0.1 n solution of $KMnO_4$) and I analyzed the phosphorus volumetrically (Pincus).

From the analytical data reported in the table it is seen that:

1) The calcium level in the blood of the guinea pigs was, after 3 weeks of daily administration of garlic juice, 2 or 3 times higher than the normal level of the controls; after 2 months of continued daily administration, it is normal and equal to that of the controls.

2) The percentual content of calcium in the ash of the bones is increased both after 3 weeks and after 2 months of administration of garlic juice, while the percentual content of phosphoric anhydride is reduced after 3 weeks and almost normal again after 2 months.

3) The percentual content of the calcium in the ash of the teeth is increased after 3 weeks and also after 2 months, whereas the phosphoric anhydride is reduced after 3 weeks and also after 2 months.

Calcemia and percentual content of calcium and phosphorus in the ash of the femurs and teeth of normal guinea pigs and those treated with garlic juice.

No. of animal	Weight of the animal Starting	Final	Days of treatment	mg calcium in 100 cc blood serum	Ash of femurs Ca%	P_2O_5 %	Ash of teeth Ca %	P_2O_5%
				Normal guinea pigs				
1	430			11.0	36.76	48.00	32.55	31.68
2	410			11.2	36.03	48.22	33.00	31.68
3	400			11.5	36.40	48.22	33.19	31.68
Average				11.2	36.39	48.07	32.91	31.68
				Treated guinea pigs				
4	400	460	21	30.0	37.54	46.34	33.95	25.60
5	390	450	21	26.0	37.70	46.00	33.40	25.00
6	395	445	21	22.0	37.70	46.34	32.40	25.90
Average				26.0	37.64	46.56	33.25	25.50
7	390	435	60	11.0	37.14	47.80	33.90	28.12
8	385	455	60	11.5	37.10	47.80	33.75	27.89
9	400	425	60	11.2	37.80	47.80	33.75	27.89
Average				11.2	37.34	47.80	33.76	27.96

On the one hand, these tests confirm the results obtained by me before on dogs with respect to the hypercalcemia produced by administration of garlic juice and hence the stimulating action which the garlic juice exerts on the function of the parathyroids. On the other hand, the present tests show that garlic juice produces a profound modification in the metabolism of the bones and of the teeth in that it increases the calcium content and reduces the phosphorus content, especially in the teeth.

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(1) This Bulletin, 1945, 20, p. 546

(Translated by Carl Demrick Associates,Inc/LH/t)

From: Deutsche Apotheker Zeitung, vol. 106, no. 51, p. 1861-1866.

Pharmacological and clinical investigations of garlic.

By Wesselin Petkov.

Garlic is one of the few plants which have been used at all times and by all nations as a valuable remedy against all kinds of diseases which have nothing in common.

In ancient times garlic was used widely in the treatment of disorders of the gastrointestinal tract. In fact, the investigations of numerous researchers (63, 39, 10a, 75, 19, 27, 18, 12 and more) proved on the basis of experiments and clinical observations that garlic has a favorable effect on the digesting function. Many pharmacologists and clinicians recognize these effects nowadays (37, 87, 82 etc.).

Among the substances which are obtained from garlic, the antibiotic "garlicin" mentioned by de Almeida Machado, Gross and Calvalhodos merits our attention. Another antibacterial substance extracted from garlic is "allicin" obtained by Cvallite and Bailey. No less remarkable are the successful efforts by Smoll, also by A. Stoll and E. Seebeck regarding the synthesis of the bactericidal garlic factors. Very interesting in this connection is ascertained the fact that allicin also has a cytostatic effect (84).

Garlic has been used most frequently in the last years for its thorougly investigated effect in atherosclerosis and hypertension; thus for example by Orzechowski and Schreiber, Hintzelmann, Roos, Schrader, Khartschenko, Piotrowski, Jummel, Rossitzkij, Loskutow, Marowa, Khakhalina, Swetchnikow etc. Recent experimental research studies by L.N. Sokolowa (1962) and the clinical investigations by S.P. Bojko (1962) of the antiatherosclerotic effect of garlic are likewise based on this assumption. Based on findings from our own experiments, which are still under way, we feel, however, that the pharmacological effectiveness of garlic is determined not so much by the readily volatile polysulfides contained therein, but rather primarily by other stable glycoside sulfur compounds.

Results of our own investigations.

In the course of our many years of studies of the pharmacodynamics of garlic, we used both native products from various parts of the plant during its different vegetation periods, and purified garlic preparations which were made available to us by phytochemists. From the abundance of data that accumulated in the course of our research, and which have been the subject of a number of publications, we will report here particularly on the antitheromatous and hypotensive action of garlic which was tested by us very thoroughly. We will also discuss the influence of garlic on the intestinal movements, its effect on microbes and parasites, as well as its properties as an antidote against chronic lead poisoning.

I. On the antitheromatous action of garlic

A. Experimental findings

1. Influence of garlic juice on the development of experimental cholesterol atherosclerosis (43).

a) Influence of garlic juice on the cholesterol level in the blood serum.

The blood cholesterol was determined with the Pulfrich photometer. The method by Urbach was so modified by us that we used 2 ml instead of only 0.2 ml serum.

We used two series of male rabbits.

The first series comprised 5 groups. The 5 rabbits of the 1st group received 0.25 g cholesterol daily per kg body weight, mixed with 2 ml cod liver oil and 1 ml garlic juice. The 3 rabbits of the 2nd group received 0.25 kg cholesterol daily mixed with 2 ml olive oil and 1 ml garlic juice. The 4 rabbits of the 3rd group received 0.25 g/kg cholesterol daily mixed with a 2 ml cod liver oil. The 3 rabbits of the 4th group received 0.25 g/kg cholesterol daily mixed with 2 ml olive oil and the 5 rabbits of the 5th group received 1 ml garlic juice daily without cholesterol. The cholesterol, olive oil and 1 ml garlic juice each were added to 25 g oats, and this feed was given to the rabbits as the first morning portion. Only after this feed ration had been used up completely, did the rabbits receive unlimited amounts of mixed

feed (oats, hay, grass, roots) but always the same amount for all. Only in two of the rabbits from group 5 was the determined amount of garlic juice diluted with 25 ml water and introduced daily with a sound directly into the stomach. Each rabbit was kept in a separate cage, but in the same room. The tests lasted 52 and 82 days, at the beginning and at the end the blood cholesterol level was determined. The rabbits received neither cholesterol, nor oil nor garlic before the second cholesterol determination. The first cholesterol determination was made after the rabbits had been kept for one week under the same conditions.

To this test series we added three control rabbits, which lived in the same room, received the same feed as the test animals, but without the addition of cholesterol, garlic and oil.

In the second test series with 14 rabbits we laid down the following test conditions; blood cholesterol determination (after the animals had been kept for a week under the same conditions); introduction of 1 or 2 ml garlic juice, diluted with 25 ml water, by mans of stomach sound; after 150 minutes we determined the blood cholesterol level again. This determination was made in both test series in all animals after a 15 hour-fast.

The tests with the first animal series showed that, in all groups which had been given cholesterol in the feed for a prolonged time, the level in the blood showed higher values than at the beginning; in the animals, however, which had received garlic at the same time, this increase was considerably less (fig. 1). The 8 rabbits of group 1 and 2, which had received cholesterol and garlic with the feed, showed an average increase from 70.8 to 558.53 mg%, the 7 rabbits of group 3 and 4 which had received cholesterol with the feed, but no garlic, showed an average increase from 70.6 to 923.8mg% under otherwise identical conditions and a test period of 66.5 days in group 1 and 2 and 63.3 days in groups 3 and 4 on the average. The 5th group (5 rabbits), which received only garlic with the feed, showed no substantial changes in the blood cholesterol level.

Fig. 1 Changes in the blood cholesterol level in rabbits which had received

only garlic with the feed (a); (b) control animals; (c) cholesterol + olive oil + garlic; (d) cholesterol + cod liver oil + garlic; (e) cholesterol + cod liver oil; (f) cholesterol + olive oil.

The results of the second test series show that a single application of garlic caused in normal rabbits no definite and essential changes in the cholesterol level in most cases.

b) Influence of garlic juice on the development of atheromatous aorta plaques.

After the completion of the tests, the rabbits of the above mentioned groups were sacrificed. The macroscopic examination of the aorta showed the following: in the rabbits which had received only garlic with the feed, as well as in the control animals the intima of the aorta was smooth, shiny and of grey-pale pink color over the entire length. In the rabbits of all groups which had received cholesterol with the feed (including those which also received garlic), the intima was not smooth. The surface showed white pin had-to pea-sized, roundish, irregular, seamy etc. elevations. Nevertheless it could be seen with the naked eye that these changes were not so pronounced in rabbits which had received cholesterol and garlic with the feed. The microscopic examination of the preparations showed likewise in individual cases that the changes were less pronounced in rabbits which received cholesterol and garlic at the same time.

2. Effect of garlic and some of the active substances obtained therefrom on blood pressure (44,47,50).

a) Acute tests on 76 cats in urethane narcosis with native garlic preparations.

When varying amounts (0.10 to 1.0 ml/kg) of freshly prepared, squeezed garlic juice, diluted with wter (1:3 to 1:100), were injected into the vena femoralis, we found an insignificant and short blood pressure reduction only with the higher concentrati ns 91:5) and (with larger quantities (0.5 to 1.0 ml/kg).

In the i.v injection of varying amounts (0.25 to 1.0 ml/kg) of freshly prepared aqueous extracts (crushing the garlic by means of glass powder and addi-

tion of water) in concentrations of 1:3 to 1:100, the hypotensive effect was somewhat more pronounced (in doses which corresponded to the same garlic weight). The greater hypotensive action of the extracts thus obtained leads to the conclusion that the cells of the garlic bulb are richer in hypotensive substances than the intercellular juices.

Native garlic preparations showed a much more pronounced effect after storage for several months, particularly after the garlic juice or the aqueous extract had been stored for 7-12 months or more. Both dried out completely in that time and turned into a vitreous, transparent structureless mass of amber-reddish color. This very mass had a great hypotensive effect after stirring with water. The i.v. injection of 0.05 g/kg of this substance (corresponding to ab. 0.20 g garlic led to a blood pressure reduction of 50 mm and more (fig. 2).

Fig. 2. Cat, 4250 g. Urethane narcosis. Blood pressure in a. carotis communis. Garlic juice (prepared seven months ago, dried out and dissolved again in water) was injected into the v. femoralis (0.20 g = 0.5 g/kg). Top curve: respiration.

b) Acute tests with hypotensive garlic factors.

Among the fractions from garlic bulbs investigated by us, some showed a considerable hypotensive effect. Thus, for example, the fraction called "allsatol" showed a considerable blood pressure reduction in a dose of 50 mu g/kg already, and in doses of 0.2 - 0.3 mg/kg a short, but great reduction of the bood pressure, followed by constant blood pressure values (somewhat below the initial level (fig. 3).

Fig. 3. Cat, 2540 g. Urethane narcosis, blood pressure in a.carotis communis. 0.65 ml (= 0.25 ml/kg) allsatol 1:1000 were injected at the arrow into the v. femoralis

Other garlic fractions showed likewise a great hypotensive effect. Characteristic of many of these fractions was the persistence of a moderate hypotensive effect up to a multiple of 10 minutes (fig. 4). The bilateral vago-

tomy had no effect on the hypotensive action of the garlic fractions obtained. The atropinization of the test animals produced a sudden reduction to a complete disappearance of the hypotensive action of the tested garlic fractions.

Fig. 4. Cat, 2330 g. Urethane narcosis, blood pressure in a. carotis communis. 0.12 ml (= 0.05 ml/kg) of substance A in a concentration of 2‰ were injected into the v. femoralis at ↓ and 2.3 ml (= 1 ml/kg) at ↑.

The pharmacological interpretation of the hypotensive action showed that the effects were primarily cholinomimetic, and that the substance affected only to an insignificant extent directly the vessels and involuntary muscles.

All garlic fractions have a great drawback: they proved unstable and their hypotensive effect diminished gradually.

c) Chronic tests on dogs with experimental hypertonia (35).

The effect of the garlic preparation allcratin, administered orally in the commercial dragee form (1 dragee twice daily) was tested on 6 dogs in which an experimental hypertonia was produced by a two-stage operative ligature of three of the four main arteries of the brain according to the method by Nastev and Popov (34). It was found that allcratin reliably reduced the increased systolic blood pressure down to values which come close to the normal values. The application of allcratin withthe preparation "Veralon" (dragees which contain additionally 1 mg total alkaloids of veratrum lobelianum) prolonged the hypotensive action.

B. Clinical results of the application of garlic preparations in hypertonia and atherosclerosis.

The first clinical observations in the treatment of high blood pressure with garlic and with the garlic preparation "Polallin" made available by us were made by K. Tschilow, L. Stantschev and Wl. Wladimirov (79).

21 hypertonics of the Clinic for Internal Diseases (Med. College Sofia) were subjected to the garlic treatment. In 5 patients the blood pressure was completely normalized. 10 showed on the average a reduction of the systolic blood pressure by 30 mm Hg and of the diastolic pressure by 20 mm Hg, and their

subjective complaints stopped spontaneously. No influence on the blood pressure was found in 6 patients in the atherosclerotic terminal stage of hypertension, except for a subjective improvement in their condition.

The above mentioned authors used the same preparation on additional 46 hypertonics, partly in clinical, partly in ambulatory treatment. In 32 cases a varying pronounced reduction of the blood pressure and subjective improvement of the condition of the patient was found.

T. Taschev, M. Chlebarowa, J. Stoilow and K. Zankow made clinical observations for 70 days on a group of 27 patients, ranging in age from 48 to 78 years, with generalized atherosclerosis, combined with more or less pronounced hypertension, who were treated with a garlic dragee preparation satal, produced according to a technology developed by us (finely ground young garlic bulbs, dried according to a special method) and Allcratin *) (Satal with addition of extr. crategi oxyacanthi sicc. and rutin). One group received 1 dragee allcratin 3 times daily, one group 1 dragee satal three times daily, and one group both preparations simultaneously in the above indicated dosage.

In most cases the subjective complaints which were due to angiospasms, such as headache, dizziness, tinnitus, varied. In 17 patients the general tonus improved. Side effects were not observed.

The reduction of the systolic blood pressure was 8 to 33 mm Hg, that of the diastolic pressure, however 4 to 16 mm Hg.

The electrocardiograms showed findings of myocardial damage, conduction disturbances (mainly of the S-heart block type) and of a previous infarct. After the treatment, the ecg of the patient remained mostly unchanged. In 5 cases the ecg was favorably influenced: four showed improvement of the ST and T-deflection, in one case the extra systole disappeared. No patient showed a deterioration of the ecg findings.

Prior to the treatment, 15 patients had normal cholesterol levels but 12 showed hypercholesteremia. After completion of the therapy, they found in 13 patients a reduction of the total cholesterol, in 5 an unchanged cholesterol level, and in 9 cases even higher cholesterol levels. While the total fats

were increased in 14 of the 27 patients prior to the treatment, only 10 showed higher values after the treatment.

On the other hand, an increase of the beta-lipoproteins and of lecithin was found in a part of the patients; as a reason, the authors suggested the possibility of a mobilization of the lipoid deposit by the therapy.

The antihypertensive effect of the garlic preparation Satal was also observed in the treatment, effected by us, of a group of workers with presaturnism (saturnism - lead poisoning). In a group of 20 workers who had been given daily for 30 days 3 x 2 Satal dragees, the systolic blood pressure values dropped on the average from 153 (± 2.6) to 129 (± 3.3) mm Hg, while in a control group with the same number of workers these values were at the start and at the end of the test period 144 (± 3.2) and 141 (±3) resp. For the diastolic blood pressure the values of the first group at the start and at the end of the test period were 90 (± 2.6) and 75.7 (± 0.49) resp. and in the control group 87 (± 2) and 86.5 (± 2.07). The difference between the mean values both of the maximum and of the minimum blood pressure before and after the Satal treatment proved significant at p less than 0.001, while the difference in the control group was not significant.

Fig. 4. Isolated intestinal loop of a rabbit after Magnus. Tyrode solution, 100 ml dish. 1 ml aqueous garlic extract (10%) injected at arrow.

Fig. 6. Test culture of staphylococcus aureus; 1-native garlic juice; 2,3 and 4 - native garlic juice mixed with medicinal charcoal and lactose; 5 - 1% chlortetracycline.

Fig. 6. Test culture of bact. dysenteriae Flexner; 1 - native garlic juice; 2,3,4 and 5 -native garlic juice in dilutions of 1:10, 1:100, 1:1000 and 1:10,000; 6 - garlic juice heated to a boil; 7,8,9 and 10 - heated garlic juice in dilutions of 1:10, 1:100, 1:1000 and 1:10,000; 11 - chlortetracycline; 12 - isotonic salt solution.

II. Influence of garlic on the peristaltic movements (45,47).

We tested various concentrations of an aqueous extract of garlic and some biologically active factors isolated therefrom on isolated intestinal loops of rabbits.

In concentrations of 1:1000 - 1:2000 the aqueous garlic extract caused regularly an increase of the amplitudes of the peristatic movements, accompanied by an increase in tonus (fig. 5).

In concentrations stronger than 1:1000, particularly pronounced in those of 1:150 - 1:250, the aqueous garlic extract causes a rapid decrease of the tonus and a decrease of the peristaltic movements until they completely disappear. Subsequent administration of acetylcholine results in a relatively short increase of the tonus, followed by a decrease. When the intestinal loop is placed in stronger garlic concentrations (1:100), acetylcholine no longer effects the complete disappearance of the muscle tone accompanied by complete cessation of the peristaltic movements.

Atropine eliminates the intensifying effect of weak garlic concentrations on the peristaltic movements. Garlic stops the spasm of the atropinized intestine, caused by barium chloride. If the isolated intestinal loop is subjected to the action of strong garlic concentrations (over 1:1000), the barium chloride can no longer restore the intestinal tonus.

These test results make the contradictory data found in the literature about the influence of garlic on the peristaltic movements understandable. Depending on the selected concentration or the dose of the selected garlic preparation, the peristaltic movement and the tonus can be either increased (by the lower concentrations) or inhibited (by the higher concentrations). An additional analysis of these garlic effects leads to the assumption that the stimulating effect on the peristaltic movements and tonus is due a cholinomimetic (parasympathicomimetic) mechanism (atropine block), while the spasmolytic effect is due primarily to involuntary muscles.

Since low garlic concentrations are formed in the digestive tracts with the usual dosage of the garlic preparations used, it can be expected that they have

a slightly tonizing effect on the intestinal musculature and improve the peristaltic movements. Such an effect, however, must be evaluated positively in the treatment of older people.

III. Effect of garlic against microbes and parasites.

In repeated tests regarding the effect of volatile and non-volatile phytoncide fractions of garlic on paramecium cultures (43), we could verify the findings of other authors (76,77,78). Assuming that no closed chamber was used, the time interval in which all paramecia were killed varied between 2 and 10 minutes (garlic juice) and 21 and 28 minutes resp. (volatile phytoncides).

We also investigated the effect of various garlic juice concentrations on the propogation of the paramecia. It was found that garlic in strong concentrations (1:10 to 1:1000) kills the paramecia, while it inhibits their propagation in weaker concentrations; in particularly high dilutions, however, (1:100,000),...it stimulates decisively the partition processes in the paramecia.

Other investigations by us (54) showed that both native garlic juice and the fractions obtained therefrom, as well as the waste product of the garlic preparation, namely the garlic oil cake, have an excellent bactericidal effect on many apathogenic and pathogenic micro-organisms, among them also on the disentery bacilli (Flexner, Sonne), on the pathogenic strain of dyspeptic bact. coli 0111, E.coli and staphyolococci. The effect of the garlic preparations against microbes was tested by us according to the diffusion method of the antibiotics in agar nutrient soils. We used both common meat-peptone agar and water blue agar. As test cultures were used freshened four-hour bouillon cultures.

It is noteworthy that heating native garlic juice, or the fractions obtained therefrom to a boil, did not reduce their antimicrobic effect, while the addition of medicinal charcoal led to their complete inactivation (fig. 6,7).

The results of our own tests justify our recommendation that garlic juice be used on a wide scale in the treatment of infectious diseases of the intestines.

IV. Garlic as a prophylactic and as a remedy in chronic lead poisoning.

The detoxifying effect of sulfur and of some sulfur compounds in lead poisoning is well known. In the course of our pharmacological studies on oleum sulfuratum (48) in tests on guinea pigs, we found that the i.m. injection of this sulfur prepration had a favorable effect on some symptoms of lead poisoning, particularly changes in weight and in the blood count. Taking into account the content of active sulfur compounds in wild ramson (allium ursinum L.) we made similar tests with an alcohol extract from bulbs of this plant (48). The ransom tincture shows a similar effect as the i.m. injection of the sulfur preparation.

The fresh juice of the bulbs of common garlic (allium sativum L.) likewise rich in active sulfur compounds, had a no less favorable effect on the course of intoxications in rabbits caused by lead acetate (extension of the life of the test animals, prevention of blood count changes) (52).

Based on these results, we made additional tests on 106 clinically healthy workers who came in contact with lead or lead compounds in their daily work. We used the preparation Satal, giving daily 2 dragees for 3 months. Under the effect of this treatment, the percentage of intoxications (spotted erythrocytes, porphyrin in the urine) dropped to a statistically significant extent. On the other hand, workers who had shown no intoxication at the start of the test, but who took Satal regularly showed neither spotted cells or porphyrin until the end of the test period. In a similar control group, which received no Satal, lead carrier findings could be detected in a statistically significant percentage at the end of a three months test period.

Similar tests, but using more recent and more accurate characteristics (blood pressure changes and quantitative porphyrin determination in the urine) were made on 40 workers in lead smelteries with ascertained intoxications. (56). Half of the selected workers received for 30 days 3 times daily 2 Satal dragees, the other 20 workers with findings of pre-saturnism were subjected to control observations.

The porphyrin content in the urine dropped on the average from 109 (±6.2) to 15 (± 2.2) gamma% in the group that received Satal. The difference between the two mean values is significant at p less than 0.001. In the control group the porphyrin content in the urine dropped from 89 (± 8.5) to 84 (± 7.7); the difference between the two means values is within the scatter range of the determination method. In a comparison of the mean values of porphyrin in the urine at the start of the test, the difference between the patients treated with Satal and the control group was insignificant at p = 0.05, at the end of the test, however, it was significant at p less than 0.001.

With regard to the cardinal symptoms of pre-saturnism, like slight anemia and spotted erythrocytes, a statistically significant influence in a favorable sense was found in the workers treated with Satal.

V. Other results of the experimental investigation of the pharmacodynamics of garlic.

In the course of tests on 30 suckling pigs we found a statistically significant stimulation of the effect of garlic on their growth. The average weight increase in 24 hours of pigs which received 1 g garlic per kg body weight with their feed, proved to be 12% higher than in the control group. Besides, the consumption of fewer feed units and digestible proteins per kg growth indicated a better utilization of the feed (55). Undoubtedly the antibacterial and antiparasitic properties of garlic play an important role in this growth-enhancing effect.

Garlic usually has a decorporating effect with regard to radioactive gold. Corresponding tests were made on white mice which received for 3 days before and after the intraperitoneal injection of 198-Au for additional 4 days garlic juice per os. The total activity of the mice (determined according to the gamma-activity of 198-Au remaining in the organism) was read 5,8,24,48, 72 and 96 hours after the injection of radioactive gold. Between the 5th and 72nd hour the accumulation of 198-Au in the mice fed with garlic was lower to a statistically significant degree than in the control mice. The easier elimination of the radio nuclide could be due to the decorporating effect of garlic.

Presumably a chemical reaction takes place between the colloid macromolecules of gold and the chemically active sulfur compounds of garlic. On the other hand, it can be assumed that the activation of the reticuloendothelium stimulated by garlic (demonstrated by us in another test series), contributes to the transformation of colloid gold into a formwhich is better suitable for conveyance and thus easier to eliminate. As evidence can be considered the lower content of radiactive gold in the spleen of rats fed with garlic in the tests.

These are the most important results of our many years of research on garlic. In addition to the verification of many therapeutic effects ascribed by folk medicine to garlic, our investigations proved that this garden plant, which is known in the entire world, has, thanks to its active biological components, other effects on the organism which are not yet recognized by the public. The clarification of the chemical nature of the versatile active substances in garlic and their isolation are of paramount importance, due to the rich pharmacodyhamics of this plant.

Summaries in German and English.

Footnote.

After the preparations Satal and Allcratin had become popular in Bulgaria after many hears of research, we learned of the German preparation "Jlja Rogoff garlic pills" by M. Woehm, Eschwege, whose formula is quite similar to Allcratin. According to the manufacturer, they also contain young garlic bulbs, finely ground and dried according to a special process, to which viscum album with its hypotensive effect (due particularly to the inhibition of the vasomotor centers of the medulla oblongata and of the spine) and extr. humuli lupuli with its mild sedative action without any side-effects have been added. The active principle of flor sophorae jap., which is also included, is rutin, which is likewise contained in Allcratin.

Translated by Carl Demrick Associates, Inc./IE/db

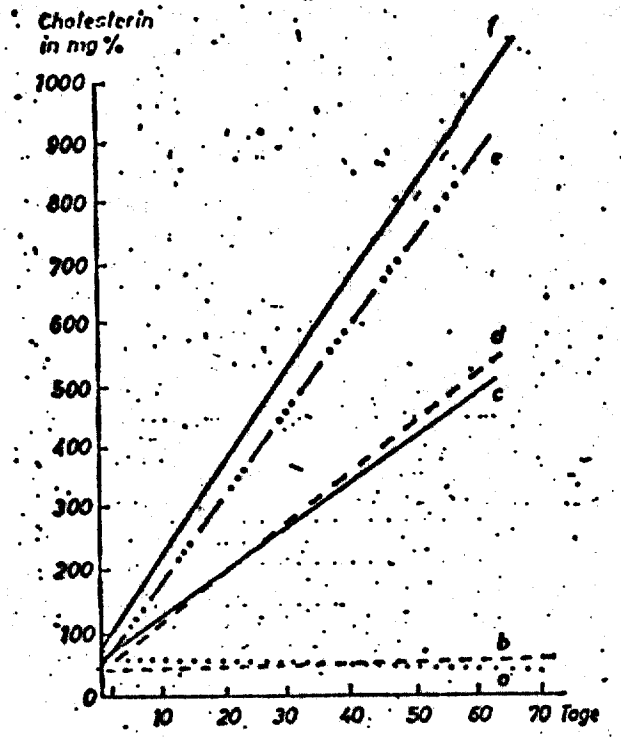


Fig. 1

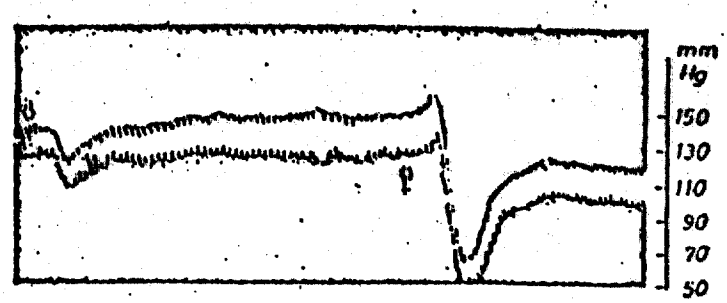


Fig. 4

Fig 5

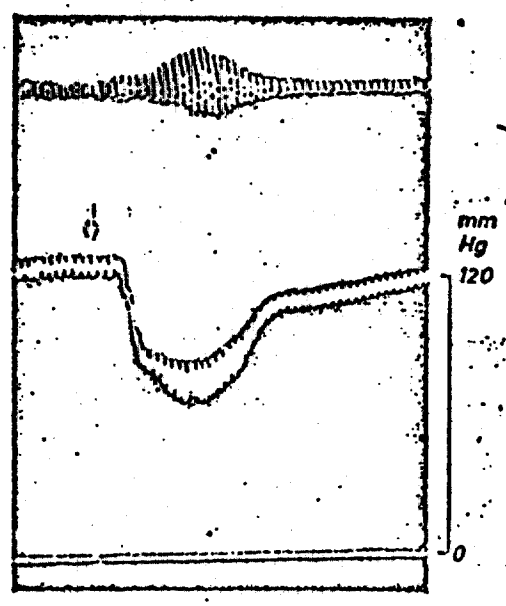


Fig 2

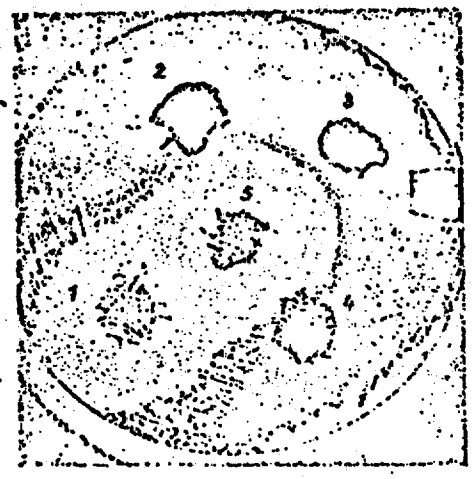


Fig 6

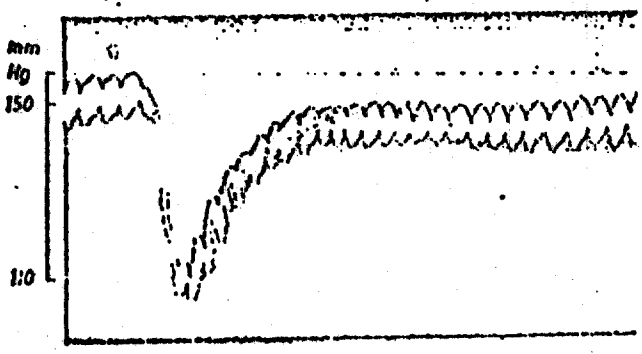


Fig 3

Fig. 7

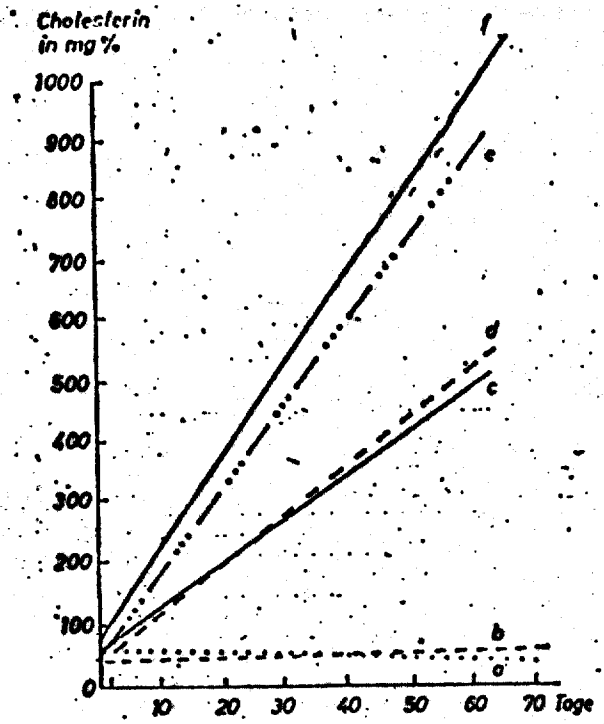
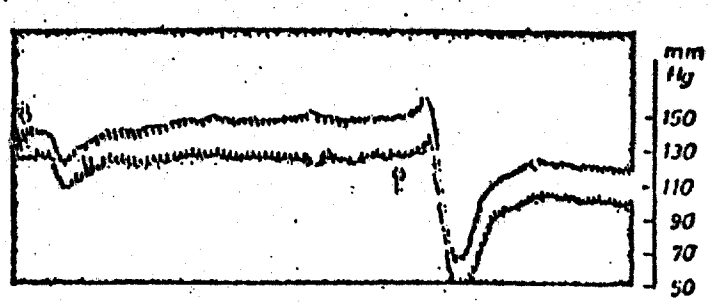


Fig. 1



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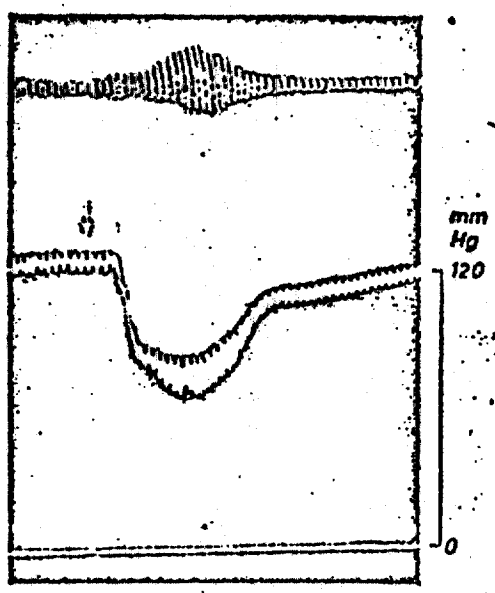

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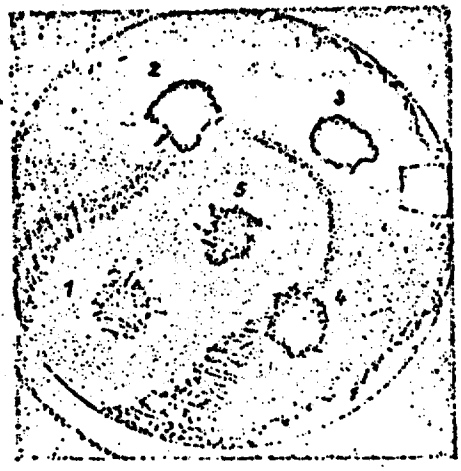

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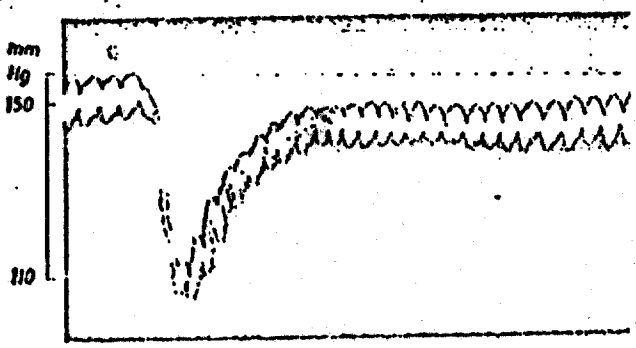

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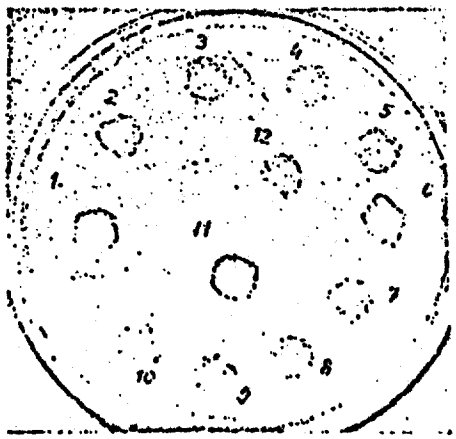

Fig. 7

zeugten in Versuchen an 75 Katzen eine markante hypotensive Wirkung.

An Hunden mit experimentell erzeugtem Hochdruck reduzierte Knoblauch den systolischen Blutdruck bis zu Werten, die dem normalen Blutdruck nahekommen. Klinische Anwendung von Knoblauch bei 114 Patienten mit deutlichem Hochdruck und Atherosklerose führte zu bemerkenswerter Beseitigung ihrer Beschwerden und in der Mehrzahl der Fälle zu einer Senkung des systolischen Blutdrucks um 8 bis 33 und des diastolischen um 4 bis 20 mm Hg.

In geringen Dosen intensiviert Knoblauch den Tonus der Darmmuskulatur und somit die Peristaltik; größere Dosen zeigen jedoch eine entgegengesetzte Wirkung.

Knoblauch übt einen signifikanten bakteriziden Einfluß auf zahlreiche pathogene Mikroorganismen aus. Erhitzung von Knoblauchauszügen bis zum Siedepunkt beeinträchtigt diese Eigenschaft nicht.

In Versuchen an Tieren und ferner in Beobachtung an Arbeitern, die Bleiintoxikationen ausgesetzt sind bzw. daran litten, wurde gefunden, daß sich Knoblauch als wirksames Vorbeugungs- und Heilmittel gegen Saturnismus erwies.

Summary

Having given a concise review of the most topical bibliographical data concerning pharmacological studies in garlic and the use of garlic preparations in the therapeutical practice, the author expounds experimental tests of his own. In tests on rabbits, he found that garlic inhibited, to a certain degree, the development of extremely severe forms of experimental hypercholesterinaemia. Garlic fractions displayed a considerable hypotensive effect (acute experiment on 75 cats).

In dogs with experimental hypertension, garlic reduced their systolic arterial tension to values nearly as high as the normal ones.

Clinical application of garlic (in 114 patients with a clear-cut hypertension and atheroselerosis) markedly improved their complaints and, in the overwhelming majority, provoked a drop of their systolic arterial tension by 8 to 33 and of their diastolic one by 4 to 20 mm of mercury. Some biochemical tests improved as well.

In small doses, garlic intensified the tonus of the intestinal muscles, thus increasing peristaltic movements, while in large doses it inhibited them.

Garlic experts a clear-cut bactericidal effect on plenty of pathogenous microorganisms. Heating of garlic to boiling point does not impair its microbicidal activity.

In experiments on animals, moreover in observations of worker groups exposed to chronic lead intoxication, it has been found that garlic is an efficacious preventive and curative drug against saturnism.

Literatur

In dem vorstehenden Beitrag, der eine gekürzte Fassung darstellt, wird nicht auf alle verwendeten Arbeiten mittels Fußnote hingewiesen. Um jedoch einen möglichst umfassenden Überblick über die Literatur über Knoblauch zu geben, seien auch die nicht ausdrücklich erwähnten Arbeiten der Originalfassung mit aufgeführt.

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Experimental study of the pharmacology of the active principles of "allium sativum" (*)

By Enrique Umbert de Torrescasana

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Introduction

A variety of properties have been attributed to "Allium sativum" and related species, and it has been used in a variety of ailments.

In many of the accredited formulas, judicious observations are mixed with crassest superstitution, but the majority of its uses (digestive disorders, respiratory ailments and circulatory diseases) have persisted over the generations, evidently showing that there is a substrate of truth, For this reason we wanted to study in detail the pharmacology of this plant, so interesting to the medical art of all ages.

According to Rungoist (45), garlic bulbs contain a sulfurized glucoside, alliin, from which, by action of an enzyme, a sulfurized essence is formed which, according to Wertheim (64), is a variable mixture of many combinations of sulfur and one of oxygen with a single allyl radical; it also contains (Semmler,49) ethylpropyl disulfide, allyl trisulfide, and probably also tetrasulfide. The active properties of garlic do not depend on the sulfur they contain (Mangrane 30). Vincent attributes them to their content of diallyl sulfides. The fresh plant contains small quantities of sulfocyanic acid (Peyer 43); Hans v. Euler and Malberg (20) have studied a very strong active principle influencial on the formation of red blood cells. Garlic contains practically no vitamin C (Jarussowa and Lanowskaya, 65); Parrino and Dominicim (41) Glasser and Drobnik (16) have related the oxidation-reduction enzymes which garlic contains with its antibacterial action, and Hietzelman has proposed a method for evaluating the garlic extracts based on its protective effect against toxic doses of irradiated ergosterol.

The antibacterial action has been studied by Dombray and Vlaniowisch(10)

Sabrazes (46), Michaelis (34), Serrel, Cooke and Gabriel (55) use garlic juice for healing purulent wounds. According to Waugh (63), the blood of subjects who have previously taken large quantities of garlic exerts a marked bactericidal action on staphylococcus cultures. Fust (15) studies the action of garlic vapors on cultures of Bang's and erysipelas bacillus and on the spores of B. subtilis and B. anthracis, and they prove that it exerts an inhibiting action.

Kolle, Laubenheimer and Volmar (25), Boecker (4), Lehmann (27), Jacobson (23), Schang (47) have also studied the inhibiting effect on other germs.

Canadell and Garcia Valdecasa (5) obtain from allium sativum extracts of great bacteriostatic activity, the active and water-soluble principle having no connection with the components of garlic essence. This principle is thermolabile, being destroyed at 50°; it is active at the concentration of 1 per million, permitting at this dilution only the growth of sixty per cent of the Flexner and Shiga. This principle does not become altered in oxygen atmosphere. It diminishes in CO_2 atmosphere.

Toroptseu and Filatova (58) observe in the treatment of infected wounds that these principles even kill protozoa; they call these principles "phytonicides." Tokin (57) recommends the use of these principles together with the sulfamides.

Cavallito and Bayley (7) observe that they act on Gram negative germs resistant to penicillin.

Since old times one admits the hypotensive action of garlic (Loeper, Debray and Pouillard, 28). It is also known to increase the energy of cardiac contraction and to reduce the frequency of the pulse.

The question of the therapeutic value of garlic and of the alliaceous products has been the object, in recent years, of many articles and controversies among the medical men (Marcovici, 31; Frenkel, Lenitzkaja, 14; Delvaille,9; Mohlen, 37; Becher and Fussganger, 1; Schlesinger, 50; Schwahn, 54; Schrader,52; May,32; Ortner, 38; Loeper and Debray,29; Roos, 44; Kretschmer, 26; Schubert,53; Shindel, 48; Noether, 36).

Also the anthelmintic action of garlic is important (Toscano Ricco, 59; Vanni, 60).

Garlic extract first excites the endocrine functions and later inhibits them, that is, small doses are stimulating, large ones inhibiting (Yun and Rhee, 66).

The toxicity of the garlic preparations has been studied experimentally by Perrin, Dombray and Vlaicovitch (42), Cavallito and Bayley (7).

EXPERIMENTAL PART

In going over the old and modern studies on garlic as we have just done, it is readily seen that there is no systematic investigation of the pharmacological action of the bulb of this plant and whether the various actions discovered in its juice (antiseptic, antidiarrhea, hypotensive, etc.) are due to a single active principle or are, on the contrary, manifestations of the presence of several pharmacological substances. For this reason, and at the suggestion of Professor Garcia-Valdecasas, we have directed the work of this Doctoral Thesis toward studying in the greatest possible detail the actions of the active principles of garlic on the various organs and apparatus of the body. Before going into our experimental study, we wish to thank Professor Garcia-Valdecasas for his constant counsel, throughout the work, and for having placed at our disposal the different chemical fractions obtained by him from the juice of the bulb referred to.

For our pharmacological study we used three chemical fractions.

1. A total extract of the garlic bulb, obtained by hydro-alcoholic extraction and concentrated, by the precipitation of the pectins, until 40 cc corresponded to one kilogram of bulbs. This extract had been tested already for its bacteriostatic properties by Garcia-Valdecasas and Canadell (5) and found to possess a high activity against the Flexner and Shiga bacilli. The mentioned authors gave this extract the name Extract 20, and we shall call it so here.

2. An extract obtained from the above by treatment with chloroform, evaporation thereof and dissolution in water, which according to the same

authors (data not yet published) also possesses a strong bacteriostatic activity. We shall call it Extract 210.

3. The solution remaining after the chloroform extraction, which has no bacteriostatic action whatever. We shall designate it as Extract 214.

What all tested extracts have in common is their intense alliaceous odor. In the following we give an account of the results of our study.

Toxicity.- We studied the toxicity in guinea pigs and rats, by parenteral as well as digestive administration. In subcutaneous administration, the three extracts proved highly irritant, easily giving rise to the formation of abscesses which interfered with the results, so that we abandoned that method of investigating the toxicity.

The toxicity by intravenous application was studied on the rat and mouse, similar values being found for both species. The LD_{50} is 0.02 cc per gram of Extract 20. The animals died quickly, after a slight convulsive period, or they survived indefinitely. However, the repetition of non-lethal doses on successive days caused death in a certain number of animals, so that a certain cumulative effect must be admitted, although we have been unable to clarify if it occurs by accumulation of substance or simply of effect.

Table 1 (Guinea pigs)

No.	4/26/45	4/30/45	5/3/45	5/7/45	5/11/45
1	620 g	550 g	620 g	603 g	575 g
2	555	420	370	abscess	
3	650	605	670	605	602
4	590	490	535	490	495
5	650	560	610	585	585
6	690	620	685	630	685
7	690	600	655	615	585
8	630	620	655	665	535
9	730	680	712	640	690
10	550	460	535	480	505

Orally the toxicity is much less. The guinea pigs weighing about 650 grams

withstand without any apparent disturbance daily doses of 1 cc (1.6 cc per kg) for an indefinite time. The administration was always be intra-esophagic sonde, to be absolutely sure of it. The records of Table 1 show a slight loss of weight.

In the rats, the results are similar. Doses of 0.5 cc given daily as indicated (5.5 cc per kg) for 15 days produced only slight loss of weight.

Table 2 (Rats)

No.	4/26/45	4/30/45	5/3/45	5/7/45	5/11/45
1	112 g	102 g	102 g	100 g	105 g
2	100	92	97	100	100
3	110	92	94	95	80 (Parturition 5/9/45
4	103	90	97	105	100
5	94	80	85	85	85
6	100	90	87	83	85
7	83	77	77	80	75
8	75	75	75	73	73
9	65	65	67	70	70
10	65	62	64	68	70

The histological study of the viscera gave the following results (1):

Spleen.- Almost without exception we found hyperplasia of the lymphoid tissue, dilation of the venous sinuses, much destruction of the red blood cells contained therein, and in some cases increase of the hematogenic pigment.

Liver.- In approximately half the animals it appears slightly congested and with presence of hematogenic pigment in the Kupffer cells. Also a slight degeneration of a cloudy or micro-vacuolar nature was seen.

Kidneys.- Slight turbid degeneration and some cases of diffused congestion. Only one animal showed frequent degenerations of a necrotic nature in the renal tubes, which were almost like the lesions produced by the mercurial poisons.

Adrenal.- Only a hyperhemic reaction manifests itself, more apparent in the medullar than in the cortical.

Comment.- We see, therefore, that the lesions are generally of a congestive nature, with degenerative tendencies in the parenchymatous elements occurring in a certain percentage of the animals.

Noteworthy is the increase of the hematic pigment clearly perceptible in many cases, suggesting a more or less strong erythrocytic action.

We found striking the inverse correlation which seems to exist between the liver and spleen lesions in the sense that the animals whose splenic lesions were more pronounced showed an almost normal liver, while those whose spleen was less altered had more intense liver lesions, greater congestion and more abundant pigment content of the Kupffer cells. The same phenomenon can be seen, although less clearly, in connection with the kidney. For example, the animal we mentioned before, whose kidney showed such grave necrotic lesions, had, in contrast, a liver and spleen of almost normal appearance.

Action on arterial pressure

The tests were always made on cats anesthesized with ether, recording the arterial pressure with a mercury manometer. The results are the following:

Extract 20.- Produces a very intense hypotensive action (Graphs 1 and 2), transitory at low doses, but very prolonged at strong doses. The constance of this reaction is absolute.

Extract 210.- As can be seen in Graph 3, it has absolutely no hypotensive action, showing, on the contrary, a slightly hypertensive action. That action was intensified as the dose increased.

Extract 214.- We see from figures 4 and 5 that this extract retains the full hypotensive capacity of Extract 20 with the same characteristics.

Comment

It follows from the above results that the garlic extract contains two completely different active principles, a bacteriostatic principle, present in Extracts 20 and 210 (Canadell and Garcia-Valdecasas, 5) and a hypotensive principle present in Extracts 20 and 214, which, as we have just shown, has nothing to do with the former. This discovery having been made, it was more interest-

ing still to study the action of both substances in the other organs. Thus, therefore, two of the effects attributed to garlic are certainly due to different substances, which can easily be separated: A predominantly water-soluble hypotensive substance, and a predominantly liposoluble antiseptic substance, although it dissolves also in water. Since almost simultaneously with the work of Canadell and Garcia Valdecasas (5) there appeared in North America the work of Cavallito and co-workers (7), who, it seems, obtained the same antiseptic substance by a different chemical method, we investigated whether said substance possessed the same pharmacological properties as those of the substance studied by our professor. In fact, the actions were absolutely identical, both as to arterial pressure and in the other organs, so that we shall hereafter use the substances extracted by either process indiscriminately in our research.

Action on isolated intestine

We used rabbit intestine prepared according to Magnus, in Tyrode's solution at 38.5°. Bath volume 100 cc.

Extract 20.- It has a typical paralyzing action, quite similar to that which we shall later describe for Extract 210, so that we shall not dwell on it here.

Extract 210.- Graphs 6,7 and 8 show the paralyzing action of this extract, better than any descriptions. The very small doses show no action (figure 6), but at a concentration of 1 x 1000 (0.1 in 100 cc) it produces a rapid reduction of movements which becomes stronger progressively and almost completely impedes the action of the acetylcholine (0.25 cc at 1 x 10,000). Stronger doses (2.5 x 1,000) produce total paralysis in a short time, and with still higher doses the paralysis is almost instantaneous.

In figure 9 we have made a careful study of the possible antagonism between the substances we are studying and acetylcholine, for as we saw in graph 6, it could be assumed. As can be seen in this figure 9, the presence of extremely small quantities of extract manifestly reduces the acetylcholinic action.

Extract 214.- The graph of figure 10 shows the effect of this extract on the isolated intestine. It is found to have absolutely no paralyzing action,

presenting, on the contrary, a slight stimulating effect, which is clearly evident from the increase in the amplitude of movements.

Comment

In their action on the isolated intestine we again see the different pharmacological action of the two active principles. The bacteriostatic principle is highly paralyzing and antagonizes the action of the acetylcholine and of other stimulants (Cl_2 Ba, etc.). This action has not been described until now by any author. On the contrary, Noether (36) described a stimulating action which in our opinion corresponds to that produced by Extract 214, namely the one which proved hypotensive in the cat. Thus, then, according to what has been reported until now, the hypertensive substance is paralyzing in the intestine and the hypotensive substance is stimulating. It seems, therefore, that the mechanism of action has some relationship with the action of the vegetative nerves. However, the hypertensive action of Extract 210 is too weak to affect a sympathetic action, and the same may be said of the stimulating action of Extract 214 on the intestine in relation to a parasympathetic action.

Action on isolated uterus

We have used the isolated uterus of virgin guinea pig, suspended in Tyrode's solution at the temperature of 38.5°. Bath volume 100 cc.

The three tested extracts are absolutely devoid of manifest action on the smooth fiber of the uterus. As can be seen in figure 11, in connection with Extract 210, the spontaneous contractions of the uterus do not change when adding to the bath 0.5 cc of Extract 210, a dose more than sufficient to paralyze the intestinal contractions very quickly. Higher doses did not show any action either. However, this inaction of the active principles of garlic on the uterus is apparent only with respect to Extract 210. In fact, we have noted the appearance, if in inconstant form, of the phenomenon represented in figure 12. It consists of the following: If a strong dose of pituitrin is added to the perfusion bath, the uterus contracts most during a certain period, after which it relaxes and then shows ample and frequent contractions. Now, if we add

Extract 210 at that moment, the original maximum contraction reappears in some cases and is maintained for a prolonged time. The phenomenon, as we have said, is not constant in all uteri, as others show, as in figure13, a progressive tendency toward atony without manifest action of the alliaceous principle.

The simultaneous addition of pituitrin and Extract 210 to the bath did not seem to modify in any way the curve which we obtained only with the first of these substances.

Action on the isolated frog heart

Lastly we though it important to study the action of these substances on the isolated frog heart, according to the technique of Straub-Fuhner, to find out whether the hypotensive action manifested in the cat was connected more with a cardiac action than with a vascular action, We did not try methods of vascular prefusion because, like that of the isolated rabbit ear, they proved impossible with the means available in the Laboratory (we had no perfusion pump), and others, like the Trendelemburg on the frog, we did not think was accurate enough.

Instead, the results obtained on the isolated heart are quite demonstrative.

Extract 210.- Graph 14 illustrates the action of this extract at very dilute doses (1 in 1,000, ½ cc) mixed approximately with another medium existing in the cannula, therefore final concentration 12n 2,000. A fairly manifest tonic action is observable, with marked reduction of the amplitude of the contractions. In a way, one may speak of a tonic action of more rapid and sustained occurrence.

Extract 214.- The action of this extract is much less intense, showing no effect at all at the dilutions mentioned above for Extract 210. In higher concentrations (1 and 2 per cent), the systolic level is reduced, as can be seen in graphs 15 and 16, which is again maintained for a long time.

Comment

The cardiac actions we have just reviewed show that the two active principles of garlic we tested have little cardiac action. Here again, the different

mode of action of the two substances appears.

Extract 210 has a clear cardiac tonic action, which may well be the cause of the slight increase in arterial tension which this extract produces.

Extract 214, on the contrary, is found to be very little active. We can safely exclude the participation of a cardiac effect on the intense hypotension which said extract produces, as the concentrations to which the heart responded with a tendency to hypodynamia (1 and 2%) can never be reached with the doses used in our experiments on arterial pressure.

We saw in them that 1 cc of Extract 214 diluted to 1 in 10 has a fairly marked hypotensive action and 2 cc of pure extract possessed an almost collapsing effect. As the animals weighed about 3 kg, their blood volume could be calculated as about 350 cc, which would give a concentration of 1 in 3,500 in the first of the above cited cases, and 1 in 175 in the second. Both doses show no action whatever in concentration in vitro.

Conclusions

1. The existence in garlic of two active substances with completely different biological effects is shown.

2. The two substances separate easily because of their different solubility in the solvents.

3. The previously described antiseptic substance in garlic is the same which produces the paralyzing effect on the intestine: it is slightly hypertensive.

4. The hypotensive substance of garlic has no connection with the antiseptic.

5. A uterine reaction difficult to interpret is described, which does not occur consistently.

(*) Summary of doctoral thesis.
Unable to Xerox Graphs

(Translated by Carl Demrick Associates, Inc/LH/t)

gamma-L-glutamyl-S-n-propyl cysteine in garlic (Allium sativum)

Artturi I. Virtanen, Margrit Hatanaka and Marja Berlin

Biochemical Research Institute of the Foundation for Chemical Research, Helsinki, Finland

In an earlier communication from this Laboratory, the isolation and characterization of gamma-L-glutamyl-S-allyl-L-cysteine from garlic was reported (Virtanen and Mattila (1). The same authors proved at least nine gamma-glutamyl peptides from garlic by fractionation with a Dowex-1 column. These are in part identical with the gamma-glutamyl peptides isolated from onions and chemically characterized by Virtanen and Matikkala (2). Thus, for example, gamma-glutamyl-phenyl alanine, gamma-glutamyl-S-methyl cysteine and gamma-glutamyl-S-beta-carboxy-beta-methylethyl-cysteinyl-glycin, which were first isolated from onion, were identified also as constituents of garlic (3).

That gamma-glytamyl-S-allyl cysteine is, however, not the only gamma-glutamyl peptide occurring only in garlic is proven by the gamma-glutamyl-S-n-propyl cysteine isolated by us from garlic. This peptide has not been found in onion, at least until now. Glutamic acid and S-n-propyl cysteine have been characterized as hydrolysis products (with 1 n.HCl) of the peptide. By paper chromatography gamma-gl.-S-propyl cysteine produced from gamma-glutamyl-S-allyl cysteine was identical with the isolated peptide.

It is odd that garlic contains propyl cysteine in the gamma-glutamyl peptide in combined form, despite the fact that in this Allium species no reduced or oxidized S-propyl cysteine has been found as free amino acid. In the onion, in which S-propylcysteine-sulfoxide is a characteristic amino acid, gamma-gl-S-propyl cysteine does not occur.

(Translated by Carl Demrick Associates,Inc/LH/t)

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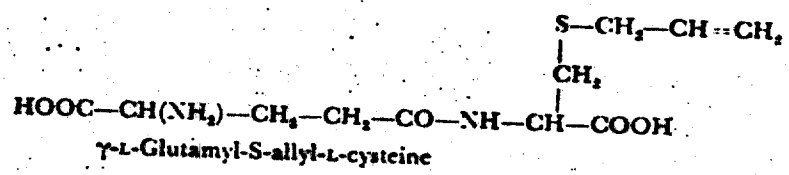
γ-L-Glutamyl-S-allyl-L-cysteine in Garlic (*Allium sativum*)

ARTTURI I. VIRTANEN and INKERI MATTILA

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

Using the same method of fractionation as Virtanen and Matikkala[1,2] in their studies on γ-glutamyl peptides in onion (*Allium cepa*), we succeeded in isolating a new γ-L-glutamyl peptide in crystalline form from garlic. The elementary composition of the peptide corresponds to the formula $C_{11}H_{18}O_5N_2S$. M.p. 156—158.5 (decomp.), $[\alpha]_D^{23}$ —17.1 (in water). The amount of the peptide isolated in crystalline form was 0.11 % per fresh weight, but its amount in garlic was noticeably larger. L-Glutamic acid and a sulphur-containing amino acid were formed from the peptide on hydrolysis with 1n HCl. The sulphur-containing amino acid took up one mole of bromine and accordingly contained one double bond. The amino acid could be established as S-allylcysteine (by paper chromatography and IR-spectrum). On the basis of optical rotation in water and in 6n HCl the isolated S-allylcysteine was the L-form. This was also the case with glutamic acid. The peptide could be reduced at 25°C with Raney nickel to glutamyl-S-n-propylcysteine. The structure of the new peptide is thus



γ-L-Glutamyl-S-allyl-L-cysteine

S-Allylcysteine does not occur in garlic in free form, but its sulphoxide is a known and physiologically important compound in garlic. It is possible, but has not been shown, that S-allylcysteine in the γ-glutamyl peptide is the precursor of the S-allylcysteine sulphoxide. The possible pathway of the biosynthesis of S-alkylcysteines *via* their carboxy compounds has been discussed by Virtanen and Matikkala[2].

A more detailed report on the work will be published in the *Acta Chemica Scandinavica*.

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Contribution to the analysis of the ingredients of garlic - Allium sativum L.(1)

Second Report (2): Gas chromatography of garlic oil

By O.-E. Schultz and H.-L. Mohrmann (3)

Numerous authors have already reported in several publications on the gas chromatographic analysis (GC) of organic sulfur compounds. Often this involves work in petrochemistry. For the present problem, only a few of the stationary phases used were suitable. Carson and Wong (1) separated synthetic organic-aliphatic sulfur compounds, some of which are also ingredients of the essential garlic oil. Also Petranek (11) examined the possibility of separating organic sulfides in the gas chromatograph. A comprehensive list of the pertinent literature is contained in the book of tables of gas chromatographs by Kaiser(7).

The gas chromatograph used for the present work is an apparatus of the firm Perkin-Elmer & Col, Model 116 E, which is equipped with a thermistor detector (WLD) and flame ionization detector (FID) (4). The recorder is a small size compensograph of Siemens & Halske having a recording width of 12.5 cm. As carrier gas helium (99.99%) was used, for the flame, electrolytic hydrogen and synthetic compressed air.

1. Preliminary experiments with garlic oil

Fresh, triturated garlic was extracted with ether. With the extract oil obtained, the following stationary phases were tested for their fitness partition for (Table 1). Also it was necessary to determine and to fix the proper experimental conditions. The data of the fractograms described below are given in table form at the end.

2. GC of the garlic oil (thermistor-detector, WLD)

2.1 For the investigation we used an oil which had been obtained by steam distillation from fresh garlic (A/D_1; see First Report, Experimental Part, 3.2). With column X (1.90 m) a division into 15 substance peaks was achieved (Fr. 15-19).

To ascertain changes in oil composition, the oil was chromatographed immediately after isolation (Fr. 17) and the analysis repeated after to 8 days (Fr. 18). An essential difference was not observable.

Table 1

Type	Stationary phase	Type	Stationary phase
I	Di-n-decylphthalate	VI	Polypropylene glycol
II	Di-2-ethylhexyl sebacinate	VII	beta, beta-oxydipropionitrile
III	Silicone oil DC 200	VIII	Squalane
IV	Polyethylene glycol 1500	IX	Dimethyl sulfolane
V	Apiezon L grease	X	Butoxyethylephthalate + tricresyl phosphate

2.2 A garlic oil (A/E) obtained by direct solvent extraction of the fresh drug (see First Report, Exp. Part. 3.1) gave in the fractogram the same number of peaks as the distilled oil. However, a comparison of the fractograms showed that the low boiling components are present in higher concentration in the extract oil, as these components are lost in the distillation to some extent.

Unlike the extracted oil, the distilled oil became dark brown after some time. This change was not demonstrable qualitatively in the fractogram. Presumably polysulfides have formed, which cannot be distilled without decomposition.

3. Tests for improving the result of separation by varying the experimental conditions

3.1 Variation of the column length

Column X was lengthened from 1.90 to 2.90 m, after which the resolution was greatly improved (Fr.24). Until then, Peak X (diallyl disulfide) had been separated from the next following Peak XI incompletely.

From measured values of this fractogram, the plate number or column length necessary for complete separation of the two substances X and XI

considered was calculated (see Section 12). For the 2.90 m column there resulted a specific separation efficiency of n_1 = 439 theor. plates/m column length at an HETP value of 2.4 mm. But the theoretical calculations gave a necessary total plate number of at least 3200.

After insertion of a column (X) of 3.60 m length, we calculated from the obtained data, using a distilled oil (A/D_1), total plate numbers of Ø 3390 with a specific separation efficiency of n_1 = 943 and an HETP value of 1.06 mm (Fr. 25; Fig. 1).

We examined whether the peaks obtained could be further resolved by the use of a column (X) of a length of 6.60 m. While the substance curves were preserved individually, they were greatly flattened due to the high retention times (Fr. 29).

Table 2

Stationary phase	Length m	Preparation	Result
1 III	2	A/D_1	Insufficient resolution (Fr.30)
2 VIII	2	A/D_1	Good resolution. Causes interference levels (Fr.32) in the FID.
3 V	2	A/D_1	Good resolution at high temperature Separation of the oils into 15 main substances (Fr. 33/34).
4 VI	2	A/D_1	Less resolution than V, otherwise same separation efficiency (Fr.37).
5 I	2	A/D_1	Result corresponding to column X (Fr. 43).
6 IV	3	A/D_1	Deficient resolution (Fr.78)
7 II, VII, IX	- 2 each	A/D_1	Unsuitable for the problem of partition.

3.2 Variation of the stationary phases

Under the ascertained experimental conditions Column V - Apiezon L - is best suited for the present problem of partition. It was possible to remedy the insufficient resolution of the low boiling components that appeared at first, by filling the injection block with silver wool to enlarge the surface.

Axial diffusion before entrance into the column was thereby greatly reduced (Fr. 36; fig.2).

3.3 Variation of temperature and carrier gas quantity

These parameters were also varied for most columns. The respective data are found in the tables of experimental data (see Section 13).

Fig. 1 Fractogram 25 Column X; 3.60 m; distilled oil

Fig. 2 Fractogram 36 Column V; 2 m; distilled oil

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4. GC of the essential garlic oil (flame detector, FID)

Working with standard analytical columns and FID makes it necessary to use a flow divider for injections of normally used substance quantities, only a small portion of the fractions that leave the column getting into the detector, while the greater part goes into the outlet, here via the catharometer (WLD). The flow conduction in the desired proportion is obtained by different flow resistances in the gas stream.

With the use of column X and operating temperatures of 150-160°C, the zero line drift due to evaporation of stationary phase was already so high because of the high sensitivity of the FID that zero matching was not attainable at high sensitivity. With column V, however, satisfactory fractograms were obtained, which were in agreement with those of the WLD (Fr. 44 to 47).

5. CG of vacuum distilled fractions of garlic oil

The fractions F_1-F_3 obtained after fractionated vacuum distillation of the oil (see First Report, Exp. Part. 3.4) were chromatographed with column V (Fr. 53_{1-3}). In like manner the fractions of the high-vacuum distillation (F_{1a}-F_{6a}) were analyzed (Fr. 54_{1-6}).

The fractograms showed that in the boiling point intervals an enrichment of certain substances had taken place. Pure substances could not be obtained by simple fractionation. Therefore, Semmler (13) could not have obtained them either in his time.

6. GC of the vapor phase of essential garlic oil

By the method described in the First Report (Exp. Part, 3.2) a fresh oil distillate (A/D) was prepared. 150 mcl of this preparation were placed in a 50-ml round flask, which could be heated by a heating plate over a paraffin oil bath. With the carrier gas (He) the vapor phase of the oil could be flushed into the 25-ml gas loop of the gas sample inlet portion via a shunt line and caused to separate after saturation by switching into the column. The metnioned flask was closed with a stopper with two bores. The entrance side consisted of a glass capillary through which helium was blown in a fine stream onto the oil surface. The outlet was connected by a plastic tube with the gas sample inlet portion of the fractometer. The scheme for the gas stream thus was: (He bottle) - Pressure reducing valve - (Shunt) - Wash bottle (H_2SO_4) - Sample flask (heatable) - Gas loop - Fractometer or wash bottle (methanol; switchable).

Chromatography on the 2-m Apiezon L column and FID. Column temperature 160°C; carrier gas quantity in the apparatus, 60 ml/min (Table 3).

Table 3

Fractogram	Oil bath temp. °C	Flow divider	Sensitivity
Fr. 56a	20	1:25	4
Fr. 56b	20	1:9	4
Fr. 56c	20	1:5.5	8
Fr. 56d	45	1:5.5	8
Fr. 56e	75	1:0.8	8
Fr.56f	20)	without	16
Fr. 56g	60)		16
Fr. 56h	65)	(= closed fracto-	16
Fr. 56k	80)	meter exit)	16
Fr. 56l	120)		16

Fig. 3 Fractogram 56k Column V; 2 m ; vapor phase of oil distillate.

For the fractograms Fr. 56f-l no flow divider was used, that is, the entire gas stream quantity went into the FID. This resulted in sufficient, recordable substance quantities also at low vapor pressure of the oil.

The fractograms Fr. 56a-e gave no characteristic picture.

Before the running through of fractogram Fr. 56_g, again 40 mol of fresh oil were charged in the flask.

At an oil bath temperature above 125°C the oil gradually darkened.

For comparison, the distilled oil was chromatographed by the method employed until then. It has been described already as Fr. 47.

The result of the test series was that all peaks obtained thus far could be obtained already at room temperature of the vapor phase, only the resolution and sharpness of the peaks is improved at higher temperature.

7. CG of the vapor phase over fresh garlic

The vapor phase over 500 g of ground fresh drug was now to be flushed directly onto the column. For this purpose, the 2-liter three-neck flask, provided with an inlet and outlet tube and KPG agitator,was connected via a "Drierite" drying tube on the discharge side directly to the gas sample kinlet section of the fractometer. As flushing gas helium was used, to avoid too great an air peak. The drug, contained in the flask, was kept in motion with the agitator. The gas path was: (He bottle) - wash bottle (H_2SO_4) - flask - drying tube - gas loop (25 ml) - fractometer or wash bottle (methanol; switchable).

The chromatographic column was again No. V at an operating temperature of 160°C. There was no flow division.

The result shows, in agreement with thin layer chromatograms (see first Report), that in fresh drug there is hardly any diallyl disulfide, mainly in favor of the easily volatile (low bioling) ingredients.

After 24 h again 300 g of fresh drug paste were charged in the flask and the vapor phase was analyzed. It was clearly to be seen that in the meantime higher boiling products (diallyl disulfide, Peak X) had formed and besides from the fresh preparation, the primary substances were prominant (Fr.58;fig.4).

Fig.4 Fractogram 58-60, Column V; 2 m; vapor phases of allium species Fr. 58, A. Sativum; Fr. 59 A. cepa; Fr. 60 A. ursinum

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8. Comparative GC of the vapor phases of Allium cepa and Allium ursinum

The vapor phases of A. cepa (Fr. 59; fig. 4) and A. ursinum (Fr. 60; fig.4) were examined in the above described test arrangement. Extensive agreement was found with the fractogram of A. satinum, although the quantitative relationships are very different. The peaks V-XI exist in them all, among them especially Peak X (dially) disulfide). While in A. ursinum the group VI/VII is pronounced the most, A. cepa contains instead a substance before Peak X which is completely absent in the other two. The strong Peak IV in A. ursinum is not found in A. cepa or A. sativum. In the latter it emerges in the distillate. This comparison showed the close relationship of these plants with respect to their ingredients.

9. Test of the GC fractions of the essential oil for sulfur content

After partition of the essential garlic oil into the individual components, the question whether at least the especially prominent main substances of the fractograms are sulfur-containing compounds was of interest.

9.1 By spot tests with ethanolic potash lye and sodium nitroprusside solution (I) and the iodoazide reaction (II) (Feigel, 3) there were examined as a preliminary analysis:

a) oil steam distillation (A/D_1)

b) oil of direct extraction (A/E_1)

c) oil of steam distillation of A. cepa.

With (I) we obtained for a-c an intensive violet color, which faded after a short time. With reaction (II) gas evolved in all samples after short shaking within 30 sec. The solutions had become colorless in 5-10 min.

9.2 Test for presence of mercaptans: 0.5 ml distilled oil was triturated with 0.2 g mercury (II) chloride and left standing for 30 min. A few ml of carbon tetrachloride were used to extract the triturate, which after elimination of the solvent was taken up in little chloroform and analyzed by gas chromatography. The fractogram showed no elimination of previously obtained characteristic peaks, thus ruling out the presence of mercaptans in larger

quantity in the examined preparation. The above test was carried out on other stationary phases with the same result.

9.3 The fractions of an oil fractogram were collected at the fractometer outlet in a sodium alkali solution of lead oxide. If mercaptans had been present, yellow lead mercaptides would have precipitated. This reaction did not occur either; only in some fractions lead sulfide formed after a prolonged time (Table 4).

9.4 Since also in hydrogen carbonate-alkaline solution a reaction with sodium nitroprusside did not occur (SH groups react positively), we charged the GC fractions in ethanol, to which a few drops of a soda-alkaline solution of potassium cyanide had been added. Three minutes after the collection of each fraction, 2-3 drops of 1% sodium nitroprusside solution were added. All tested samples gave a positive reaction by showing a more or less strong violet color (opening of thio-ether and disulfide bonds, Table 4).

Table 4

Peak	Plumbite solution	Alkaline KCN solution
I/II	-	-
III	dark brown	+
V	dark brown	++
VI	dark brown	++
VII/VIIa	dark brown	+
VIII	dark brown	++
X	dark brown	++
XI	dark brown	++
XII	dark brown	+
XIII	-	+
XIV	-	+

Table 5

Peak	Iodoazide reaction (min)
I/II	10
III	at once
V	2
VI	2
VII/VIIa	at once
VIII	1
X	at once
XI	at once
XII	at once
XIII	3
XIV	3

9.5 Somewhat higher concentrations of the collected substances in either solution were added to 2 ml iodoazide reagent, and the time to decoloration of the solutions was measured (Table 5). In agreement with the preceding experiment, one obtains positive results here again for Peaks III-XIV, so that sulfur compounds must be present in them. Besides, the collected substances all have an odor typical of such compounds. The difference in the decoloration times is, however, not only a result of the catalytic acceleration by a compound type (thio ether, disulfide, etc.) but is also dependent on concentration.

10 Experiments on identifying the GC-separated substances

Synthetic commercial diallyl disulfide was chromatographed on Column VI. The main peak coincides exactly with the retention time of Peak X from the garlic oil, which there constitutes the major portion of the components. This test was carried out with the same result on the other stationary phases. This substance was thus identified in the fractogram (Fr. 70).

10.1 As another test substance synthetic n-propylallyl sulfide was used and co-chromatographed. The substance peak does not coincide with any of the

garlic oil, so that the presence of this compound as an oil component is ruled out (Fr. 73).

10.2 Three more peaks of the oil fractogram were identified in the above described manner by the corresponding authentic compounds of the market. The compounds dimethyl sulfide, dimethyl disulfide and diallyl sulfide could thus be correlated with their peaks as oil constituents.

10.3 The retention values of dimethyl trisulfide and diallyl trisulfide were, to begin with, determined graphically from the values of the homologous sulfides and disulfides. For this reason, the retention times were plotted on a half-logarithmic scale against the number of sulfur atoms (fig.5).

—— Fig.5 Dependence of log t_{dr} on the number of S atoms

A Diallyl-mono-di-trisulfide B dimethyl-mono-di-trisulfide

The values obtained from the intersection with the S_3 ordinate each corresponded to one of the peaks present in the oil fractogram. Thus these substances had to be the compounds sought.

The investigation was carried out on three stationary phases of different polarity (Squalane VIII; Apiezon L V; Polypropylene glycol VI), with the same result. The A curves belong to the allyl sulfides, the B curves to the methyl sulfides (Fr. 79, 82, 83).

10.4 To verify the above obtained theoretical result, dimethyl and diallyl trisulfides were produced synthetically (see 10.5) and chromatographed. The theoretically ascertained and the experimentally found values were identical, and the substance peaks in the oil fractogram were thus determined. Table 6 gives the experimental results for the identified substances.

Table 6 Retention times and relative retention values (referred to diallyl disulfide) of the identified substances.

Peak	Column V t_{dr}	V_{rel}	Column VIII t_{dr}	V_{rel}	Column VI t_{dr}	V_{rel}	Substances
I	1.35	0.056	1.65	0.049	1.20	0.051	Dimethyl sulfide
IV	4.20	0.17	5.35	0.16	3.95	0.17	Dimethyl disulfide
V	6.65	0.28	9.60	0.28	6.35	0.27	Diallyl sulfide
VIII	16.0	0.66	19.60	0.58	14.0	0.59	Dimethyl trisulfide
X	24.05	1.00	33.80	1.00	23.55	1.00	Diallyl disulfide
XIV	85.8	3.56	116.0	3.44	80.1	3.41	Diallyl trisulfide

10.5 To synthesize dimethyl trisulfide and diallyl trisulfide, 2 g each of the disulfides were addedwith 0.4 g of elementary sulfur and 0.4 g of di-n-butylamine as catalyst. After letting stand for 3 hours in a water bath maintained at 30°C, little dilute hydrochloric acid was added, and after thorough shaking the sulfides were extracted with ether. The ether solution was dried over sodium sulfate, freed from solvent to a large extent, and chromatographed.

The preparation of sufficient quantities of the individual fractions with the analytical columns used by us by frequent repetition of the analysis was not possible with the purity and in the quantity required for substance analyses.

11. Test for substance decomposition during the GC analysis

11.1 Fava et al. (2) and also Olander (10) have investigated by gas chromatography the effect of ionic shift by mercaptide ions in mercaptan-disulfide mixtures under thermal or metal catalysis according to the scheme:

$$RS^{(-)} + R' - S - S - R' \longleftrightarrow R - S - S \quad R' + R'S^{(-)}$$
$$\downarrow$$
$$R - S - S - R + R'S^{(-)}$$

It easily occurs upon normal distillation of such mixtures; for diallyl disulfide it was observed in the presence of zinc with formation of the monosulfide, metal sulfide and polymer.

In the garlic distillate to be examined, mercaptans are not perceptibly present in the native state; they would have been eliminated with mercury chloride as mercaptide. On the other hand, there remains the possibility of thermal decomposition of the oil ingredients, possibly under the influence of

a metal catalysis of the fractometer material.

11.2 Carson and Wong (1) have separated mixtures of pure substances of 15 aliphatic, symmetrical and asymmetrical (C_1-C_5)-di- and tri-sulfides. They have determined the effective and relative retention volumes (referred to cyclohexanone).

Under the operating conditions used, they are able at the same time to rule out decomposition of the substances during the analysis by examining the collected fractions. Their operating temperature was 150°C and the evaporation temperature 170°C. Rate of flow: 50 ml/min helium.

11.3 Under analogous conditions with the use of cyclohexanone as inner standard on the Apiezon column, the relative retention volumes were not reproducible in the magnitude of the stated values (Fr.74). In addition, the silver wool was temporarily removed from the injection block, in order to preclude any catalytic influence. This did not result in any difference in the total fractogram.

11.4 As another comparison with the work of Carson, Column IV was used. However, it was unsuitable for the present problem because of insufficient resolution. The relative retention value found for diallyl disulfide was 2.01, in contrast to that of 1.87 found by Carson (Fr. 78/78a).

11.5 Of the oil F_{3a}, using column V, 2 times 8 mcl were collected in chloroform after passage through the fractometer. The collected fractions were examined in parallel for comparison with the oil F_{3a} by thin layer chromatography (see first Report). The chromatograms were completely identical, thus ruling out decomposition (Fr. 79).

12. Calculation of some column characteristics from measured values

The concept of the plate number was extended by Martin and Synge (8) from the processes of counter-current distribution to chromatography columns.

A number of authors have since then dealt with the calculations of this parameter and have developed formulas concerning it (Mayer and Tompkins, 9; James and Martin, 6; Glueckauf, 4; Hrapia and Leibnitz, 5; and others).

The plate height must be regarded as a characteristic quantity of a chromatographic column. A "trennstufe" or theoretical plate (5) (HETP) is defined as a zone of length L in which an equilibrium has adjusted itself between the mean concentrations of the substance in the gas phase and the mean concentrations in the stationary liquid phase.

Calculation of the plate heights from measured values of a fractogram thus offers the possibility to evaluate the separation efficiency of a chromatographic column.

In one of the following examples it is very clearly evident, however, what deviations occur in the end result if the theoretical considerations of the various authors are taken into account. Moreover, the substances here examined are not non-polar substances which are generally used on non-polar phases due to the compensation between molecular bond forces for the determination of theoretical relationships.

12.1 Purnell (12) states a formula for the calculation of the plate height which correctingly takes into account the diffusion in the gas phase.

$$n = 8 \times \text{in } 2 \; \frac{t_{dr} \times t_r}{(b'/2)^2}$$

n = number of theoretical plate equivalents

$t/$ (min) = retention time (stay in the liquid phase)

t_d (min) = break-through time (stay in the gaseous phase)

t_{dr} (min) = total retention time

n_1 = separation efficiency = plate number/m column length

$b'/2$ (mm) = measured half-height width of a substance band

$(t_{dr} = t_d + t_r)$

With this formula the plate height for the 3.60 m column (X) was calculated from the fractogram (Fr. 25). The first peaks were not taken into account here, as they fall outside the average value.

Table 7

Peak	t_{dr}	t_r	b'/2	n
V	14.0	13.0	5.8	3000
VI	22.6	21.6	9.0	3340
VII	24.8	23.8	10.0	3270
VIII	34.2	33.2	13.0	3770
X	57.3	56.3	23.5	3180
XI	61.3	60.3	25.0	3280
XII	86.7	85.7	32.5	3890

ø plate number 3390; separation efficiency n_1 943/m; HETP 1.06 (mm).

12.2 In analogous manner the plate heights applicable for the Apiezon column (V) were calculated from the fractogram (Fr. 79; fig.6) according to various formulas employed for these calculations (Table 8).

Fig. 6 Fractogram 79 Column V; 2 m; F_{3a}

Formula I (See p. 445)

Table 8

Measured values

Peak	t_{dr}	t_d	b'/2	$\sqrt{t_{dr}(t_{dr}-t_d)}$	b/2	V_{dr}	beta
III	28	6	1.5	22.48	1.15	1476	18.7
IV	40	6	2.0	36.82	1.65	2111	20.0
V	62.5	6	3.2	59.4	2.85	3299	19.5
VI	94.5	6	4.6	91.4	4.25	4980	20.6
VIII	150	6	7.5	147	7.15	7920	20.0
X	229	6	11.0	226.2	10.65	12100	20.8
XI	256	6	13.0	253	12.65	13510	19.7
XII	357	6	15.5	353.9	15.15	18860	23.0

Plate numbers ø = 20.3

Peak	Formula I n	Formula II n	Formula III n
III	1930	3290	2582
IV	2215	3260	2785
V	2341	2670	2410
VI	2339	2750	2570
VIII	2219	2440	2345
X	2399	2600	2543
XI	2149	2270	2215
XII	2932	3090	3030
Ø	2316	2770	2560
n_1	1158	1385	1280
HETP	0.86	0.72	0.78

Table 9 Relative retention values

Test conditions				Peak (v_{rel})														
Col.	Length m	Temp. °C	He ml/min	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIIa	XIII	XIV
I	2.00	160	64.0	0.065	0.084	0.11	0.16	0.26	0.40	0.45	0.60	-	1.00	1.15	1.51	-	-	-
IV	2.00	150	50.0	-	-	0.11	-	0.24	0.43	-	0.68	-	1.00	-	-	-	-	-
V	2.00	160	52.8	0.065	0.085	0.12	0.18	0.28	0.42	0.48	0.66	0.83	1.00	1.12	1.57	-	2.36	3.56
V	2.00	160	64.0	-	0.084	0.13	0.18	0.28	0.42	0.46	0.65	0.87	1.00	1.11	1.57	-	2.32	3.56
VI	2.00	160	69.0	-	0.084	0.13	0.18	0.28	0.42	0.46	0.65	0.87	1.00	1.11	1.57	-	2.32	3.56
VIII	2.00	160	52.0	-	0.079	0.13	-	0.29	0.41	0.45	0.60	-	1.00	1.13	1.47	-	-	-
X	1.90	155	58.0	-	0.086	0.10	0.16	0.24	0.39	0.44	0.61	0.80	1.00	1.09	1.53	1.70	2.51	-
X	1.90	154	65.0	-	0.11	0.13	0.19	0.24	0.39	0.44	0.61	0.78	1.00	1.09	1.54	1.76	2.56	-
X	2.90	154	65.0	-	0.072	0.10	0.14	0.22	0.39	0.40	0.61	0.84	1.00	1.08	1.52	1.70	2.51	-
X	2.90	170	52.0	-	0.093	0.12	0.21	0.26	0.40	0.45	0.62	0.79	1.00	1.06	1.51	-	2.39	-
X	3.60	160	68.0	0.052	0.089	0.10	0.16	0.25	0.40	0.44	0.62	0.77	1.00	1.09	1.52	-	-	-
Identified peaks				+			+	+			+		+					+

Table 10 Test data

Fr. #	Stationary phase	Col. 1 length m	Temp. °C	Evap. temp. °C	pH	ml/min	Detector	Remarks
15-19	X	1.90	154	260	0.6	65	WLD	
24	X	2.90	170	200	0.7	52	WLD	
25	X	3.60	160	270	1.0	68	WLD	
29	X	6.60	160	252	2.0	44	WLD	
32-82	VIII	2.00	160	268	1.3	54	WLD	
33.34.36	V	2.00	160	268	1.2	64	WLD	
37	VI	2.00	160	268	1.12	69	WLD	
43	I	2.00	160	268	1.2	64	WLD	
44.45	X	1.90	154	260	pHe 0.6 pH_2 0.5 pair 1.0	65 16 260	FID	
46.47	V	2.00	160	268	1.2 pair 0.6 1.3	60 21 345	FID	
531-6	V	2.00	160	270	1.0	52.8	WLD	
541-6 56a-e	V	2.00	160	gas phase	1.2 0.6 1.3	60 21 345	FID	with flow division
58.59.60	V	2.00	160	gas phase	1.3	345	FID	w/out flow division
70	VI	2.00	160	268	1.2	69	WLD	
73.74.75	V	2.00	150	180	1.0	52.8	WLD	
78.78a	IV	2.00	150	180	1.0	50	WLD	
79	V	2.00	160	268	1.0	52.8	WLD	
83	VI	2.00	160	250	1.0	52.8	WLD	

Formula II: $n = 8 \times \ln 2 \dfrac{t_{dr}^2}{(b'/2 - v''/2)^2}$

Formula III: $n = 8 \times \ln 2 \dfrac{t_{dr}\ (t_{dr} - t_d)}{(b'/2\ - b'''/2)^2}$

b'/2 measured half-height width; b"/2 measured air peak half-height width;

b"'/2 graphically determined correction factor (detector vol. - diffusion; fig.7)

b"/2 b"'/2; b/2 - b"'/2; V_{dr} retention volume;

beta separation factor = t_{dr} : b/2.

Formula III of Hrapia and Leibnitz (5) was modified to the form of the conventional GC nomenclature. An oil (F_{3a}) was analyzed. In Table 8 are listed the plate numbers calculated by means of the various formulas side by side for comparison. The restrictions to be made in view of the polar nature of the substances apply also here.

A comparison of the fractograms Fr. 25 and Fr. 79 shows that the substances X and XI could not be resolved better by the use of a column of only about 950 plates of specific separation efficiency than was possible with one of about
(sic) 1200 plates. This latter plate number had therefore been necessary for the partition problem.

Fig.7 Graphic determination of b"'/2 (Fr. 79)

It can be seen from the values for V_{dr} that almost 19 liters of helium were required up to the emergence of Peak VII.

In Table 8, the deviations at the lower and higher retention values are clearly to be seen. This phenomenon is in agreement with theoretical considerations also from other sectors of chromatographic methods.

13. Tables

Table 9 gives the relative retention volumes obtained. They represent mean values from several fractograms of equal test conditions. The retention times of Peaks I and II could not be unambiguously determined in all cases.

Table 10 contains the test data for the individual fractograms.

Summary

There were examined the conditions for the gas chromatography of the ingredients of the essential oil of Allium sativum L.

As constituents in addition to diallyl disulfide (cf. also first Report) (6), the following compounds were identified: Dimethyl sulfide, dimethyl disulfide, diallyl sulfide, dimethyl and diallyl trisulfide. Mercaptans could not be demonstrated in a native state.

Comparative analyses with oils from Allium cepa L. and Allium ursinum L. showed extensive agreement in qualitative respect while the quantitative relationships were clearly different.

Literature

(Omitted)

Received December 28, 1964

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(1) Dedicated to Prof. Dr. W. Poethke on his 65th birthday.

(2) First Report: Pharmazie 20, 379 (1965)

(3) Part of Dissertation, Kiel 1962/63.

(4) We thank the Deutsche Forschungsgemeinschaft for supplying the equipment.

(5) Plate equivalent

(6) Pharmazie 20, 379 (1965)

(Translated by Carl Demrick Associates,Inc/LH/t)

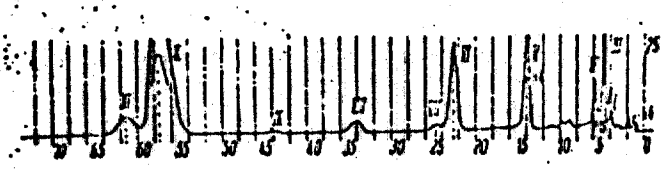


Fig. 1

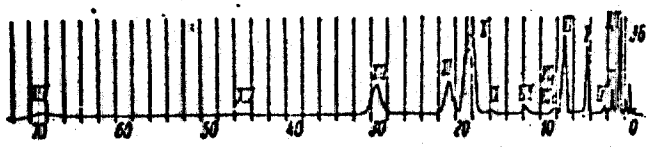


Fig. 2

Fig. 3

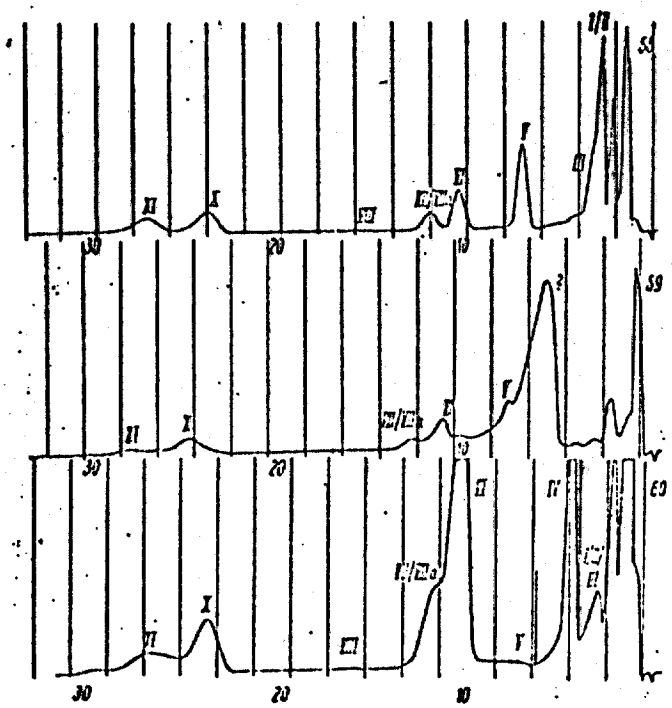


Fig. 4

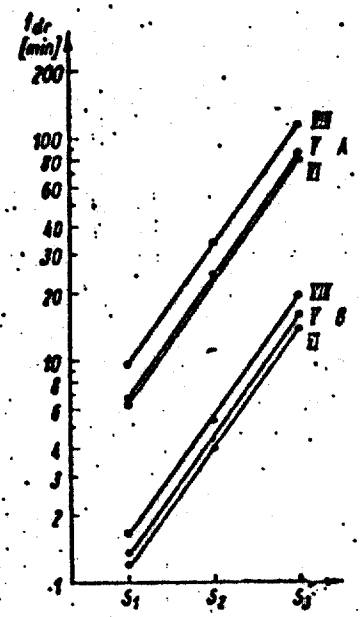


Fig. 5

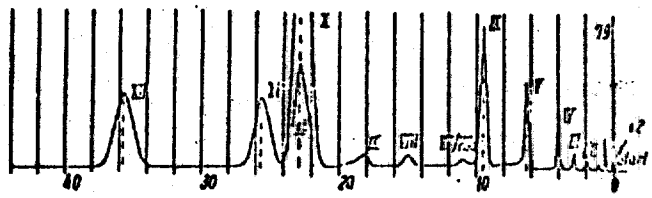


Fig. 6

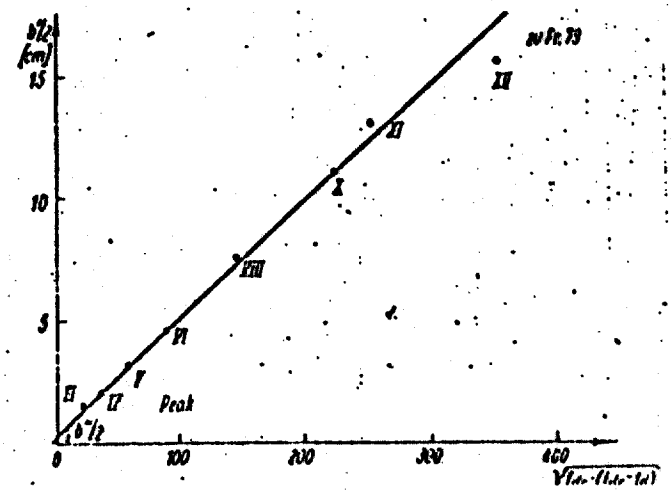


Fig. 7

Institute of Pharmacology, University of Munich, and Research Institute Pharmacy and Biochemistry, Prague

Value determination of garlic preparations

First Report: Chromatographic studies on the native ingredients of Allium sativum L.

By L. Horhammer, H. Wagner, M. Seitz and Z. J. Vejdelek

1. Introduction

Most of the preparations produced today for therapeutic purposes from garlic (Allium sativum) are more or less purified extracts which are said to contain allicin as the main active substance. The content of active substances can be checked microbiologically. In addition there exists a chemical method developed by Jager (15) based on the photometric determination of the pyroracemic acid formed from alliin by alliinase action. In this enzymatic degradation, 1/2 mole allicin is formed along with 1 mole pyroracemic acid and 1 mole ammonia. The purpose of our experiments was to see if this chemical determination can replace the microbiological control, or respectively whether the results obtainable with both methods can be brought into agreement with one another.

In this connection it seemed to us desirable to verify the results obtained until now on the ingredients of garlic.

2. Ingredients of garlic known until now

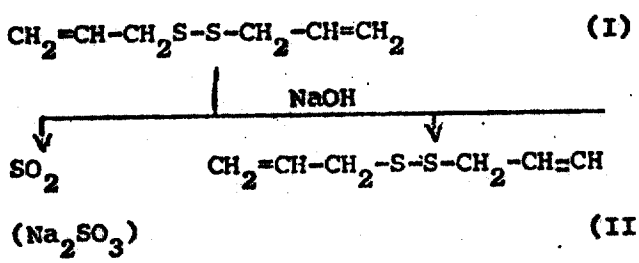
The essential oil volatile with water vapor, the odor-carrying substance of garlic, consists predominantly of a mixture of aliphatic mono- and poly-sulfides (26, 45), of which diallyl disulfide must be regarded as the main product. The composition of the onion oil obtained in the same manner (from Allium cepa(18)) is similar. More recent analyses, carried out by gas chromatography, come from Schultz Mohramm (25). Table 1 gives a survey of the sulfides found until now in these oils.

The data on the quantities of the various organic sulfides in these essential oils vary according to origin and harvest time of the tested bulbs and according to the method of processing. Semmler (26) and Wertheim (45) give as composition for garlic oil, forexample, 60% diallyl disulfide, 20% diallyl

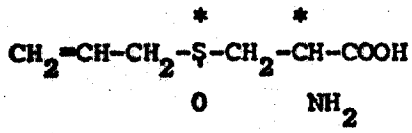
trisulfide and 6% propylallyl disulfide in addition to minor quantities of diallyl tetrasulfide and diethyl disulfide. But the allyl sulfides consistently always form the bulk.

In analogy to the mustard oils, it was assumed for a long time that the odor substances of the allium species resulted from enzymatic lysis from little odorous or entirely odorless, higher molecular, native substances. This assumption was confirmed in 1944 by the work of Cavallito et al. (8-10). The authors were able to isolate from an aqueous-ethanolic garlic extract by steam distillation under reduced pressure an unstable, water-soluble oil (8, 9), which they called allicin. For this compound there was later determined the structure of an allyl thiosulfinic acid allyl ester (9, 10, 32). As microbiological testing showed, allicin may be regarded as the main carrier of the bactericidal properties of garlic. It is, however, a disadvantage that allicin is subject to gradual decomposition. Upon prolonged storage of the comminuted drug in air, upon its steam distillation, and by action of alkalies, there is formed from the allicin (I), in addition to some diallyl polysulfides, mainly the diallyl disulfide (II) according to Scheme 1.

Scheme 1



After it was established that allicin is not native in the drug (10), Cavallito et al. (10) were able to show in 1945 that in the garlic an amino acid was contained in a concentration of 03 - 0.4%, which was broken down by enzymes to allicin. Two years later, Stoll and Seebeck (32) were able to isolate this amino acid in the pure state. They called this amino acid alliin (III). Its structure was determined in 1948 as an S-allyl-cystein sulfoxide (27-29).



(III)

Of the four possible optical isomers, alliin proved identical with (+)-S-allyl-L-cystein sulfoxide (27-29). Alliin itself constitutes an odorless, water-soluble, rather stable but antimicrobially completely ineffective substance. By the enzyme alliinase contained in garlic it is soon degraded to allicin, pyro-racemic acid and ammonia being also formed (Scheme 2).

Table 1

Sulfides	Found in Allium sativum	Found in Allium cepa
Monosulfides		
Dimethyl sulfide	(25)	-
Diallyl sulfide	(3,12,24,38)	(6)
Di-n-propyl sulfide	-	(11)
Disulfides, symmetric		
Dimethyl disulfide	(24)	(5,14)
Diethyl disulfide	(26,45)	-
Diallyl disulfide	(11,24,26,45)	(11)
Disulfides, asymmetric		
Methylpropyl disulfide	(24)	(5,14)
Methylallyl disulfide	(14,24)	-
Propylallyl disulfide	(24,26)	(6)
Trisulfides		
Dimethyl trisulfide	-	(5)
Dipropyl trisulfide	-	(5)
Diallyl trisulfide	(26,45)	-
Methylpropyl trisulfide	-	(5)
Polysulfides		
Diallyl tetrasulfide	(26)	-
Diallyl polysulfides	(26,45)	-

Table 2

Type	Allicin		Alliin		Desoxyalliin	
Chem.designation	S-alkylthiosulfinic acid ester		S-alkylcystein sulfoxide		S-alkylcystein	
Structure	R-S-(O)-S-R		R-S-CH_2-CH(NH_2)-COOH		R-S-CH_2-CH(NH_2)-COOH	
R	A. sat.	A. cepa	A. sat.	A. cepa	A. sat.	A. cepa
Methyl-	-	-	33, 34	35, 41	33	22
Ethyl-	-	-	-	-	-	-
n-Propyl	-	-	-	22, 35 39, 44	37	39
Allyl	6, 8, 9	-	9, 10, 17, 32-34	neg. 39	30,36, 37	neg.39

This mechanism of lysis is the basis of the photometric determination of allicin proposed by Jager (15). The pyroracemic acid released in the lysis is reacted with dinitrophenyl hydrazine, and the dye solution is determined photometrically.

In 1961, Japanese schientists found that besides alliin there occurs in the drug also S-allyl cystein - or desoxyalliin (37). This finding is significant insofar as here for the first time a cystein sulfoxide derivative was found in a plant together with the analogous desoxy derivative. These findings were confirmed by later investigations (33, 36). At the same time the presumption was expressed that desoxyalliin is possibly the biogenetic precursor of alliin (37). If this presumption is correct, there results for the genesis of the allyl derivatives in garlic mentioned until now the following sequence:

Desoxyalliin - alliin - allicin - diallyl sulfides

3. Original studies on the ingredients of garlic

If the diallyl sulfides are indeed formed according to the above scheme, it may be assumed that also the other dialkyl sulfides contained in the garlic oil were formed in this way and must have analogous prior stages in the drug. Since the garlic oil contains methyl, ethyl and n-propyl sulfides, it should

be possible to demonstrate in the drug also the corresponding methyl, ethyl and n-propyl analogues of desoxyalliin, of alliin, and of the secondarily formed allicin. TAble 2 shows which of these analogues have already been found in garlic and in onion.

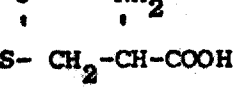
In another synopsis (Table 3) are stated the amino acids which are chemically related to the compounds mentioned in Table 2 and which have also been found in garlic or onion.

The mode of formation of all identified sulfides by the stated scheme is suggested also by the studies of Stoll and Seebeck (30) on the enzyme alliinase. These authors were able to show that the enzyme is not alliin-specific.

Table 3

Amino acid	Allium sativum	Allium cepa
Cystein	-	(22)
Cystin	(2)	(22)
Methionine	(17, 33, 37	-
Methionine sulfoxide	(33)	(22)
Cycloalliin	(33)	(16, 20, 40-42)
Other S-containing amino acids	(34)	

This lysis is evidently undergone by all cystein derivatives of the type



in which R is an aliphatic - and unsaturated - chain, but not aryl. The amino group must not be substituted. Also, the sulfur atom must be present in the form of a sulfoxide, and of the two hydrogen atoms of the methylene group in beta position only one may be substituted. The only specificity of the alliinase enzyme action is that only derivatives with the optical configuration of the natural alliin /(+)-S-allyl-L-cysteinsulfoxide/ are broken down easily and quickly. The lysis of the (-)-S-allyl-L-cystein sulfoxide proceeds more slowly and the isomers derived from the D-cystein are not broken down by alliinase(30).

As was to be expected, accordingly also the n-propyl derivative (dihydroalliin) is broken down into n-propyl-thiosulfinic acid n-propyl ester and one mole pyroracemic acid, as Scheme 3 shows. (See p. 464)

To find out whether still other derivatives occur in the drug besides the stated amino acids or S-alkyl cystein sulfoxides that can be broken down by the alliinase action to form pyroracemic acid, among others, we isolated from fresh garlic bulbs the amino acid mixture according to Stoll and Seebeck (32) and examined it by thin layer chromatography on Silica Gel G Merck. We obtained the best separation in the solvent system n-butanol/n-propanol/glacial acetic acid/water (3:1:1:1). We were thus able to separate the mixture into seven ninhydrin-positive substances (A-G, fig.1). To render the amino acids visible, we used a ninhydrin reagent containing collidin and copper nitrate. This method of identification has the advantage of permitting a color differentiation of the sulfoxides from the cystein derivatives. The sulfoxides burn orange, while the other amino acids take on a violet color. The methods described in the literature (7, 13, 23) for the chromatographic differentiation of the sulfoxides from the other amino acids were no improvement over ours. Of these seven substances, D and G (fig.1) were alliin and desoxyalliin. A comparison with synthetic compounds proved the identity.

F

Fig. 1

AG = Amino acid mixture; 1 = Alliin (synthesized); 2 = Desoxyalliin (synthesized); 2 + 1 = Mixture of 1 and 2 (1:1).

To identify the other spots, we synthesized five additional cystein and cysteinsulfoxide derivatives. Their chromatographic distribution in the system n-butanol/n-propanol/glacial acetic acid/water (3:1:1:1) can be seen from the schematically illustrated chromatograms (fig.2 and 3). The alkylcystein sulfoxides occupy the lower and central R_F region, while the alkyl cysteins are distributed in the upper region. To determine the identity of the individual compounds of our amino acid mixture with the synthetic amino acid derivatives,

we proceeded as follows. We applied the amino acid mixture AG several times successively on a 20x20 cm plate and admixed each time one of the six synthesized compounds in the ratio 1:1.

The substances of the natural mixture which are identical with one of the synthetic compounds are recognizable by a clearly intensified reaction due to superposition.

Fig.2 S-alkylcystein sulfoxides

1 = Allyl; 2 = Methyl; 3 = Ethyl; 4 = i-propyl; 5 = n-propyl; 6 = n-butyl

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Fig. 3 S-alkylcysteins

As fig. 4 shows, with the exception of n- and iso-propylcystein sulfoxide, the mentioned substance overlap occurred with all applications; n- and iso-propyl-cystein sulfoxide furnish together a new spot previously not existing in the amino acid mixture, proving their absence in the drug examined by us. We could not identify the spot A, which certainly does not represent a cystein or cystin.

Fig. 4

Fig. 5
AG = Amino acid mixture; AAG = AG after action of alliinase; AL = Alliin; AAL = Alliin after action of alliinase.

To prove in still another way that the substances B, C, D and E actually are alkylcystein sulfoxides, we incubated the amino acid mixture with purified alliinase. When using a twice purified enzyme preparation, the orange spots B, C, D and E had disappeared almost completely after action of alliinase for 5 minutes. Instead there appeared at the former sites only very faint violet spots. The same picture resulted from an alliin which had been broken down under the same conditions (fig.5). It has not yet been possible to clarify whether the remaining faint spots are due to optical isomers more difficult to break down which were still native in the mixtures.

We confirmed our findings by converting the components of the amino acid mixture with dinitrofluorobenzene into the N-dinitrophenyl derivatives according to Levy and Chung (13) and analyzing the DNP mixture by paper chromatography (4). The absence of the methyl and ethylcystein sulfoxides and of the desoxyalliin and S-methylcystein was thus confirmed.

On the basis of these experiments we were able to prove that several S-alkylcystein sulfoxides are contained in garlic. Six of the seven chromatographically demonstrable amino acids were identified. These are: S-methylcystein sulfoxide (substance B), S-ethylcystein sulfoxides (substance C), S-allylcystein sulfoxide (= alliin; substance D), S-n-butylcystein sulfoxide (substance E), S-n-methylcystein (substance F) and S-allylcystein (= desoxyalliin; substance G). Of these compounds, S-ethylcystein sulfoxide and S-n-butylcystein sulfoxide were demonstrated for the first time in Allium sativum; S-n-propylcystein sulfoxide (dihydroalliin) was not to be found in the drug available to us(*). The fact that the two newly found cystein sulfoxides show the same rate of enzymatic lysis as alliin suggests that they have the same configuration and that, therefore, this is (+)-S-ethyl-L-cystein sulfoxide and (+)-S-n-butyl-L-cystein sulfoxide.

4. <u>The problem of alliin and allicin determination according to Jager (15)</u>

It has been shown by this research that garlic contains at least four S-alkylcystein sulfoxides, all of which furnish pyroracemic acid besides the corresponding thiosulfinates. As Jager's method is based on the chemical determination of this keto acid, it determines in the drug all other structure analogues together with alliin. Besides it has been observed that the allicin analogues formed by enzymatic lysis from the other S-alkylcystein sulfoxides have very little biological activity. For this reason the results of this photometric determination cannot be in agreement with the microbiological tests.

Jager's method of chemical determination is therefore not suitable for determining the actual alliin and allicin content in garlic and its preparations. It cannot replace the microbiological quality test of garlic preparations.

5. Experimental part

5.1. Isolation of an amino acid mixture from garlic (AG) (27)

250 g of fresh garlic bulbs of Yugoslav origin, frozen with solid carbon dioxide and finely cut up, were extracted first with 820 ml and then with 480 ml of 80% methanol; we evaporated the combined filtered extracts until dry and dried them for 3 h at 70-80°C and 10 mm Torr to constant weight. We dissolved the obtained crude product (23 g) in 90 ml water while stirring and added 600 ml ethano in the course of 10 min. Then, after storage in the refrigerator for 12 h, we decanted and evaporated the solution until dry. The light-yellow hygroscopic evaporation residue (10 g) was digested with 180 ml of ice-cold methanol, the whole kept at 0°C for 2 h, suctioned off, the precipitate washed with a total of 150 ml of ice-cold methanol and afterward also with 70 ml of anhydrous ether and dried under vacuum (0.1 mm) at 150°C for 2 h. The residue is a white powder (2.5 g),which is no longer hygroscopic. According to the photometric determination - ninhydrin method according to Moore and Stein (21) - this mixture (AG) contains 54.2% amino acids (calculated as alliin). For purification, 1.5 g of this AG mixture were dissolved in 3 ml of 40% ethanol and applied on a column of 46 g silica gel (0.05-0.02 micron, column of 25 mm in idameter and 460 mm in height). We eluated with 40% ethanol and collected fractions of 5.2 ml each. Fractions 1-10 were free from amino acids (total 690 mg), 12 (180 mg), 13 (140 mg), 14 (100 mg), 15 (50 mg), 16 (20 mg) and 17 (10 mg). These fractions were combined (680 mg) and the amino acid content was again determined photometrically with the ninhydrin method (98.80% calculated as alliin). A separation of the individual amino acids was not achieved with this method.

5.2. Thin layer chromatography

The amino acids were dissolved in 40% ethanol and chromaographed on p lates Silica Gel G Merck 10 x 20 and 20 x 20 cm. The solvent system that proved best was the mixture n-butanol/n-propanol/glacial acetic acid/water (3:1:1:1). For detection the following reagent was used: Solution A: 100 ml of a 0.4% ninhydrin solution (in anhydrous ethanol) are mixed with 20 ml glacial acetic acid and 4 ml

2.4.6-collidin; Solution B: 1% solution of $Cu(NO_3)_2.3H_2O$ in anhydrous ethanol. Before use, 50 ml of Solution A and 3 ml of Solution B are combined. With this reagent the sulfoxide derivatives are stained orange, the alkyl cysteins violet.

5.3. Synthesis of alkyl cysteins and alkyl cystein sulfoxides

5.3.1. S-methyl-L-cystein

By reaction of the cystein hydrochloride in aqueous-ethanolic solution with methyl iodide in the presenceof sodium hydrogen carbonate according to Zahn and Traumann (47). M.p. 240-242°C (from dilute ethanol). $alpha_D^{20}$ - 21.5° (H_2O).

5.3.2. S-ethyl-L-cystein

From cystein hydrochloride and ethyl bromide in aqueous-ethanolic 2n NaOH according to Armstrong and Lewis (1). M.p. 240-242°C (from aqueous ethanol). $alpha_D^{20}$ - 23.8° (H_2O).

5.3.3. S-n-propyl-L-cystein (dihydrodesoxyalliin)

By action of n-propyl bromide on aqueous-ethanolic solution of cystein hydrochloride in 2n NaOH according to Yurugi (46). M.p. 242-243°C (from 30% ethanol). $alpha_D^{20}$ - 25.8° (H_2O).

5.3.4. S-isopropyl-L-cystein

From cystein hydrochloride and isopropyl bromide in aqueous-ethanolic 2n NaOH according to Stoll and Seebeck (31). M.p. 234-236°C (from 75% ethanol). $Alpha_D^{20}$ -24.1° (H_2O).

5.3.5. S-n-butyl-L-cystein

By reaction of n-butyl bromide with L-cystein hydrochloride in aqueous-ethanolic 2n NaOH according to Armstrong and Lewis (1). M.p. 227-229°C (from dilute ethanol) $alpha_D^{20}$ - 19° (H_2O).

5.3.6. S-allyl-L-cystein (desoxyalliin)

Prepared from L-cystein hydrochloride via the $HgCl_2$ complex by reaction with allyl bromide according to Stoll and Seebeck (27). M.p. 218-219°C (from 50% ethanol). $alpha_D^{20}$ - 15.2° (H_2O).

5.3.7. S-alkyl-L-cystein sulfoxides

The sulfoxy cystein derivates were obtained according to Stoll and Seebeck (31) and according to Yurugi (46) as follows: 2 g of S-alkyl-L-cystein were dis-

solved in 35 ml of glacial acetic acid, the solution was added with 1.6 ml of 30% H_2O_2 solution and left standing at 10-12°C for 8 h, the solution was added with animal charcoal, filtered, and the filtrate evaporated until dry under vacuum at a maximum bath temperature of 50°C. The dry residue was then crystallized out of 70-85% acetone (derivatives: methyl,ethyl, propyl, iso-propyl, butyl) or respectively from 50% ethanol (allyl). The pure sulfoxy derivatives exhibited the following properties. Methyl derivative: M.p. 161-162°C. $Alpha_D^{20}$ - 3.7°. Ethyl derivative: M.p. 166-167°C. $alpha_D^{20}$ - 5.1°. n-Propyl derivative (dihydroalliin): M.p. 161-164°C. $alpha_D^{20}$ - 11.2°. Isopropyl derivative: M.p. 156-157° C. $alpha_D^{20}$ - 13.5°. n-Butyl derivative: M.p. 158-160°C. $alpha_D^{20}$ - 12.1°. S-allyl derivative (alliin): M.p. 148-149°C. $alpha_D^{20}$ - 8.9°.

5.4. N-DNP amino acid derivatives

5.4.1. Preparation

The preparation of the DNP derivatives from the synthetically produced S-alkyl cystein derivatives as well as from the amino acid mixture AG was effected according to Levy and Chung (19) in sodium carbonate solution with 2,4-dinitrofluorobenzene. The excess reagent was removed with ether, the clear solution acidified, and the precipitated DNP derivatives either suctioned off (in the case of the S-alkyl-L-cystein sulfoxides) or extracted in ether (for the S-alkyl cysteins).

5.4.2. Thin layer chromatography

The experiments were carried out on silica gel GF_{254} Merck. The mixture chloroform/glacial acetic acid (70:5) was used for developing. Under these conditions the sulfoxy derivatives remained close to the starting line (R_F 0.03). The S-alkylcystein-DNP derivatives had R_F values between 0.35 and 0.46.

5.4.3. Paper chromatography

Whatman Paper No. 1 was used. The paper was impreganted with 40% formamide solution in ethanol with addition of 5% ammonium formate. Developing was done with chloroform. In this flow chromatogram (15 h) the sulfoxy-DNP deriv-

atives remained at the start, while the S-alkylcystein-DNP derivatives separated well. The spots can be recognized in daylight by their inherent color. The separation of the sulfoxycystein-DNP derivatives could be achieved by developing in a mixture of n-butanol/glacial acetic acid/water (4:1:5). In this solvent system the S-alkyl-cystein-DNP derivatives migrate with the front, while the sulfoxides distribute themselves separately over the entire R_F region.

5.5. Enzymatic lysis tests

Portions of 0.2 ml of a 0.1% solution of the amino acid in 50% ethanol were added with 5.0 ml of alliinase solution (30), heated for 10 min at 35 to 37°C in a thermostat, and 0.01 ml of this solution was then used for thin layer chromatograph.

We thank Dr. K. Macek for carrying out the paper chromatographic and thin layer chromatographic analyses of the DNP derivatives of the amino acids. Mr. Z. J. Wejdelek thanks the German Academic Exchange Service for the grant of a 3-month stipend, whereby the execution of the present investigations was rendered possible.

Summary

In order to establish whether the chemical value determination of garlic proposed by Jäger can replace the microbiological determination, garlic extracts were for the first time examined by thin layer chromatography for the occurrence of sulfur-containing amino acids.

Besides S-methylcystein sulfoxide, S-ethylcystein sulfoxide, S-allylcystein sulfoxide (alliin), S-n-butylcystein sulfoxide, S-n-methylcystein and S-allylcystein (desoxyalliin), there were identified as new amino acids S-ethylcystein sulfoxide and S-n-butylcystein sulfoxide by comparison with synthetic compounds. The various sulfoxides exhibit the same alliinase lysis rate as alliin and should, therefore, possess the same optical configuration. Since besides alliin also the other amino acids furnish pyroracemic acid after action of alliinase, and since the various allicin analogues have only a very slight biological activity compared with allicin, the microbiological quality test of garlic preparations cannot be replaced by Jäger's method.

Literature

(Omitted)

(*) To what extent origin, harvest time and storage influence the qualitative and quantitative composition will be the subject of further research.

Translated by Carl Demrick Associates, Inc./LH/db

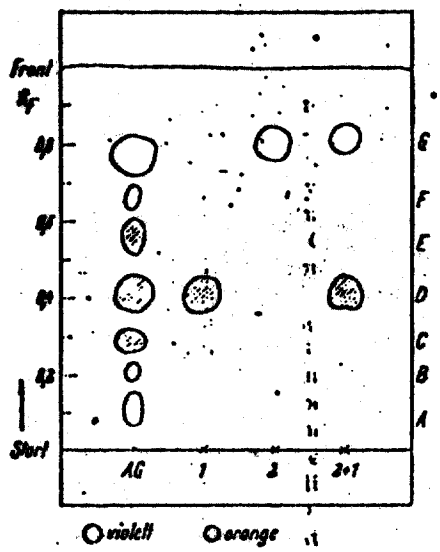


Fig. 1

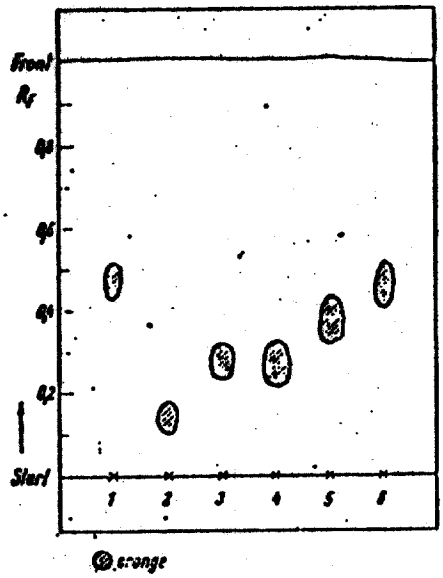


Fig. 2

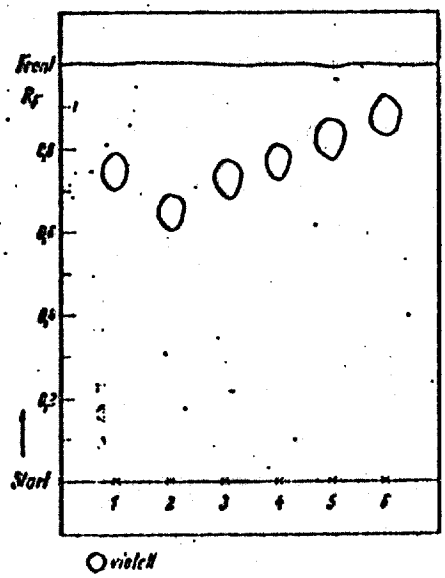


Fig. 3

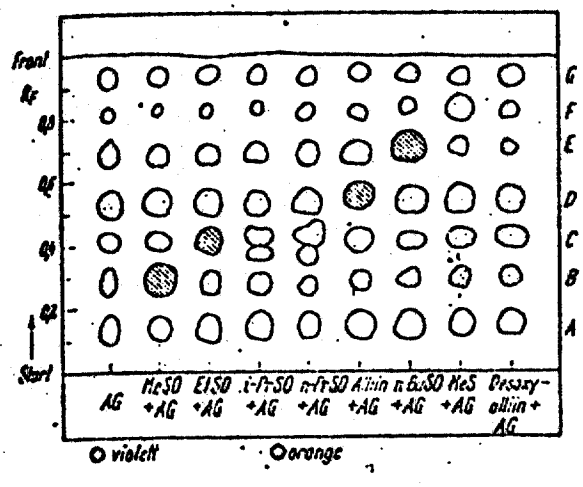


Fig. 4

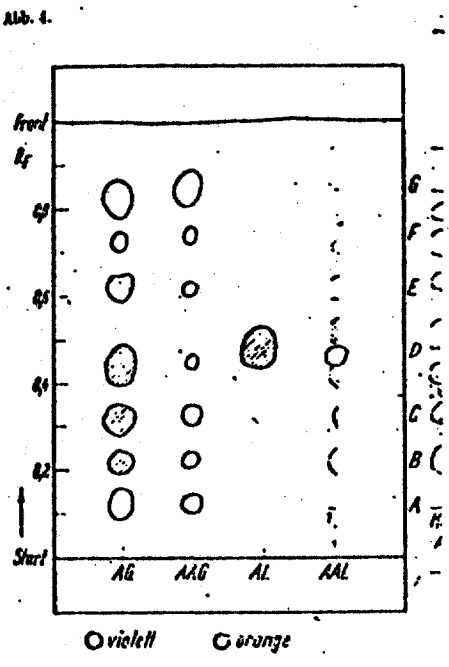
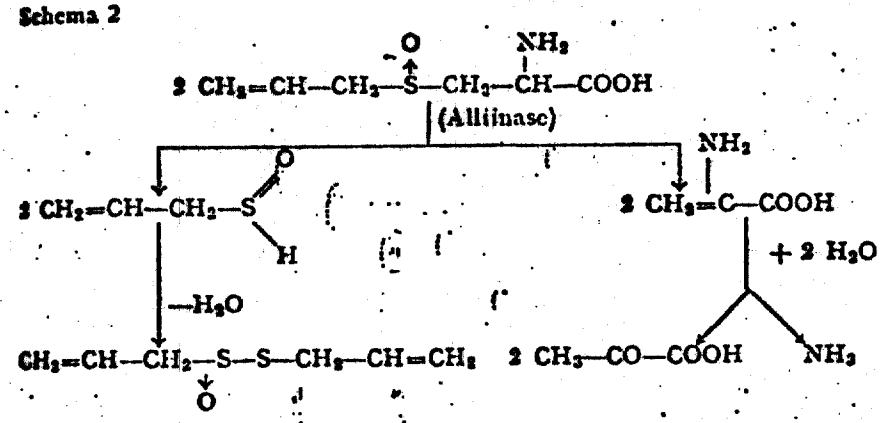
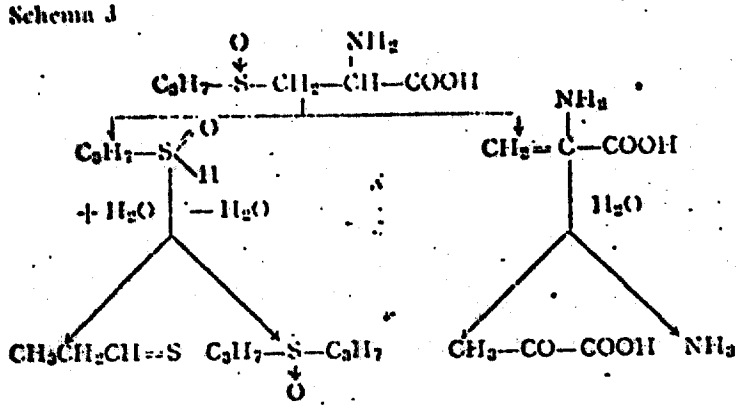


Fig. 5

Schema 2



Schema 3



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EFFECTS OF DIETS CONTAINING FOODS WITH POSSIBLE ANTIBIOTIC PROPERTIES ON THE GROWTH AND DEVELOPMENT OF WHITE RATS

By ANNANELL C. JUBB, DENA CEDERQUIST, RUTH M. NITCHALS, MARGARET A. OHLSON

SECTION OF FOODS AND NUTRITION

MAN HAS SOUGHT CURES for his ailments in the plant life around him from time immemorial. The herb doctor of medieval ages was regarded highly, and our grandmothers kept herb gardens in the interest of the family's health. Several modern drugs have been isolated from plant tissue, and, although the former faith in many of the remedies has been discredited, unexplained curative powers also have been demonstrated.

The possible connection of epidemic diseases to season and to the foods generally available seasonally should not be overlooked. Goldberger (6) and Spies (31) have demonstrated this relationship to pellagra in the southern states.

The use of food in improving the health of populations has progressed rapidly with the knowledge of the structure of the proteins and vitamins and the function of these and other compounds found in food. A casual survey of the literature makes it clear that many unidentified food factors exist. It is possible that certain of these factors may be important in the control of infections. Feeding trials of materials of suspected antibiotic potency are pertinent, therefore, in order to determine the effect on growth and function as well as resistance to infection. The former is the purpose of these studies.

EXPERIMENTAL PROCEDURES

Litter mate, weanling, albino rats from the same stock (Wistar strain), weighing an average of 55 grams, were placed in individual screen bottom cages, and given a semi-synthetic diet and tap water *ad libitum*. Five males and five females were used for each experimental garlic diet and a total of six males and six females maintained

ARTICLE 30-20

TABLE 1—*Composition of the experimental diets*

Diet	Cereal substance	Ingredients					
		Casein	Yeast	Salt mixture	Corn* oil	Cod-liver oil	Garlic
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Control I	Cornstarch 64.0	18.0	8.0	4.0	5.0	1.0	
2.5% Garlic	Cornstarch 61.5	18.0	8.0	4.0	5.0	1.0	2.5
5.0% Garlic	Cornstarch 59.0	18.0	8.0	4.0	5.0	1.0	5.0
Control II	Cornstarch 64.0	20.0	6.0	3.0	6.0	1.0	
Barley	Wholegroundbarley 76.0	11.0	4.0	3.0	5.0	1.0	
Corn	Wholegroundcorn.. 72.0	15.0	4.0	3.0	5.0	1.0	

*Wesson (38).

as controls. There were four males and four females fed each of the grain diets and the comparable control diets. The composition of the diets is shown in Table 1. The control diets used in this study were semi-synthetic. While they contained all known requirements for rat growth, it was hoped that optimal reproductive performance would not be obtained so that either accelerating or depressing effects of the test foods could be measured.

Incorporated with the diet just before feeding, was 2.5 and 5 percent of dehydrated garlic,[1] prepared from mature cloves. These quantities were equivalent to 10 and 20 percent of fresh garlic. Grains used in the diets were Michigan barley, test weight 46 to 48 pounds to a bushel, and No. 2 yellow corn. These were purchased in quantity by the College for stock and poultry feed. After grinding, the grains

TABLE 2—*Weight gains and utilization of food by first-generation animals*

Sex	Group	Number of animals	Mean weight			
			At weaning	Gained in 200 days	Of young reared	Of tissue produced/gm. food eaten
			gm.	gm.	gm.	gm.
Male	Control	6	52	392	.....	.111
	2.5% Garlic	5	61	335	.....	.107
	5.0% Garlic	5	55	308	.....	.099
Female	Control	6	55	195	363	.155
	2.5% Garlic	5	54	204	165	.122
	5.0% Garlic	5	53	175	302	.153

[1]Prepared by the Basic Vegetable Products Company, Vacaville, California.

TABLE 3—*Weight gains of second-generation animals*

Group	Mean weight			
	At weaning		Gain in 287 days*	
	Male	Female	Male	Female
	gm.	gm.	gm.	gm.
Control	59	57	227	197
2.5% Garlic	52	50	209	186
5.0% Garlic	50	48	221	195

*Mean length of life. All were mated until young were produced or until it was evident they were sterile.

were sifted through a coarse sieve to remove husks and bits of cob, and ground again to pass a small mesh kitchen sieve. Each set of experimental diets was calculated to contain comparable amounts of the known nutrients.

The animals were weighed weekly, and food consumption and weight gains are recorded in Tables 2 and 7.

Examinations of the bloods were made when the rats had been fed the diets for 4 weeks, and at 30-day intervals thereafter for a total of 6 months. Blood from a tail clip was collected in a paraffin cup containing heparin. All sampling was done in duplicate by two of the authors[2] at approximately the same time of day. Hemoglobin was determined by the method of Sheard and Sanford as adapted for use in the photo-electric colorimeter (11). Blood for the red cell counts was taken in certified Thoma pipettes and diluted with Hayem's solution. The pipettes were shaken for 3 minutes and sampled in certified Neubauer Improved Hemacytometers. Chambers were checked for uneven distribution and eliminated before counting if irregularities of filling were evident. The average value of four chambers was recorded as the cell count.

When the animals were 4 months old, brother and sister rats on the same diet were mated. Males were left with the females for 10 days, then the females were bedded on wood shavings until the young were weaned. Females were remated one week after removal of the first litter of young. First litters were discarded.

Representative second-litter, second-generation rats were placed on the parental diets at weaning, and brother and sister rats mated at maturity. After the second litters were weaned, the parent animals

[2]A. C. J. and R. M. N.

were sacrificed and complete gross autopsies were performed. When the second-generation animals had reared their young, or when it was evident that they were sterile, they were sacrificed and autopsied.

GARLIC

For centuries medicinal properties have been attributed to plants of the botanical genus "Allium." Recently workers report curative powers of garlic or onion in isolated cases of dyspepsia, gastro-enteritis, and celiac disease (19, 24), diabetes (18), typhoid fever (20), and tuberculosis (20). A controversy flared briefly over the control of arterial hypertension with a garlic extract, "allisatin" (22, 28, 33). Frenkel and Lewitzkaja (4) found that 3 percent of garlic in a diet caused a significant reduction in intestinal putrefaction in rats, and several workers record the bactericidal effects of the juice, vapors, and extracts of garlic (12, 37), and onions (5, 16, 23, and 36). Carl and his co-workers found that a diet containing 20 or 30 percent of raw garlic would kill adult rats in 3 to 11 days, but they did not describe the pathological changes which occurred (2). "Onion poisoning" and anemia have been reported in cattle (7), dogs (10, 26, 27), and horses (35) but the effects on the blood picture have not been noted after feeding garlic.

This study was undertaken to determine the effects of the ingestion of large quantities of garlic on the blood formation, growth, and reproduction of white rats.

Results

It will be noted in Table 2 that male rats fed the control diet made the best total growth, but also ate the most food. Those fed 2.5 percent garlic gained more than those fed 5.0 percent garlic although food intake was the same.

Since the female rats were allowed to reproduce, the weight of the litters at weaning was calculated as tissue produced. All females produced more tissue per gram of food consumed than the males. Seemingly, 2.5 percent of dry garlic permitted the least tissue growth as the young of this group were lighter in weight at weaning.

Although the mean trend of hemoglobin values in male and female rats fed these diets was higher when no garlic was fed and progressively lower as the concentration of garlic increased, these differences were found to be insignificant when the "t" test was used to compare the groups. Erythrocyte counts for the animals followed the same

TABLE 4—*Hemoglobin concentrations and erythrocyte counts for rats of the first-generation on the experimental diets*

Sex	Group	Hemoglobin grams 100 ml.		Erythrocytes million c.m.m.	
		Mean	S.D.	Mean	S.D.
Male	Control	15.66	.593	8.94	.342
	2.5% Garlic	15.00	.614	8.81	.570
	5.0% Garlic	14.39	.336	8.44	.552
Female	Control	15.44	1.36	8.53	.497
	2.5% Garlic	14.63	1.11	8.01	.490
	5.0% Garlic	14.03	.841	7.76	.790

pattern as the hemoglobin values, but again differences were insignificant. Garlic powder in the dietary of albino rats equivalent to 10 and 20 percent of the fresh bulb appears to have little effect on erythropoiesis. All the values were within the ranges reported by Donaldson (3) and by Reich and Dunning (25).

A summary of the reproductive performance of the stock colony for a 10-year period is included with the data for the experimental animals in Table 5. Although the females fed the control diet produced as many young per animal as stock colony females, only 42 percent of them were reared. Garlic-fed rats bore fewer young, but reared more of them, comparing well in this respect with the stock animals. The rats fed 2.5 percent of garlic showed the best results for the number and percentage of young reared, but the young weighed

TABLE 5—*Reproductive performance of two generations of rats fed the experimental diets*

Group	Generation	Litter number	Number of females		Young		
			Mated	Bearing litters	Total born	Percent reared	Mean weight at 28 days (gm.)
						%	
Control	1	2	6	6	50	42.0	46.2
	2	1	6	2	8	25.0	62.0
2.5% Garlic	1	2	5	5	30	73.3	42.0
	2	1	5	3	14	72.0	44.3
5.0% Garlic	1	2	5	5	33	63.6	45.1
	2	1	4	0			
Stock						66.1	51.0

TABLE 6—*The incidence of lung lesions in two generations of rats as revealed by gross autopsy*

Sex	Group	Lungs	
		Normal	Abnormal
		Number of rats	Number of rats
Male	Control I	6	5
	2.5% Garlic	5	5
	5.0% Garlic	5	4
	Stock	3	14
Female	Control I	7	5
	2.5% Garlic	5	6
	5.0% Garlic	3	6
	Stock	12	57

less at weaning. All young in the experimental groups weighed less at weaning than did the young of the stock colony rats, even though fewer young were reared by each female.

Greenwood (8) reports that stock animals reared 73.6 percent of their litters to a weaning weight of 48.8 grams (28 days of age). Slonaker (29) fed a diet containing an amount of protein similar to that of these studies to female rats who reared 60.3 percent of the young, with a weaning weight at 25 days of 27.5 grams.

The second-litter young were fed the parent's diet after weaning and brothers and sisters mated when 6 months old. Only one-third of these matings were successful, although the rats were given additional alphatocopherol and those males and females who failed to intermate were mated with stock animals of known fertility. Fewer young were born to second-generation females on all diets than to the parent animals, but the females fed 2.5 percent of garlic reared the same percentage of their young as the parents. These young weighed less than the young of the control group. The 5-percent garlic diet failed to support reproduction in a second generation of albino rats.

Careful autopsy revealed the lung congestion common to laboratory rats in 42, 40 and 50 percent of the control, 2.5-percent garlic, and 5.0-percent garlic diet animals (first generation), respectively, and in 45, 64 and 63 percent of their young, respectively. The lesions varied from a red, congested appearance to nodules of caseous material. Parent male animals were more severely affected than the females, but the reverse was true of their young. Slightly more males were affected in the stock colony. This agrees with the data of

Slonaker (30) who found that the male rats in his studies were more susceptible than the females. Since this lung condition is known to involve a secondary invasion of bacteria, garlic did not exert an anti-biotic effect under the conditions fed.

Summary—Garlic

A powder made from whole garlic cloves was incorporated into a semi-synthetic diet for albino rats, and its effect on growth, reproduction, and the formed elements of the blood was observed.

Although garlic caused a slight depression in the growth of male rats, the addition of 5 percent dry garlic to the diet of female rats permitted the production of more tissue than did the addition of 2.5 percent of dry garlic. The offspring of rats fed 5 percent of dry garlic powder were sterile.

There was a slight but not significant depression in hemoglobin concentrations and erythrocyte counts when garlic was fed. However, the values for garlic-fed rats are within the range reported by other workers.

Female rats of both generations fed 2.5 percent of garlic reared the largest number and percentage of their young, but the weight of the young at weaning was less than in the other groups. The weaning weights of the young of females on all three diets were lower than those of the young of stock colony females. Thus the control diet proved to be a valid one against which both decreased and increased growth performance could be measured.

The ingestion of garlic did not inhibit a chronic lung congestion in these animals, and its incidence was increased in the second generation of garlic-fed animals.

BARLEY AND CORN

There are no references in the literature to support the assumption that barley has anti-biotic properties, but because of the persistence in folk-lore of the use of barley-water for intestinal upsets in infants (21), and studies of its superiority as a ration for laying chickens (14 and 34) and for rats (32), and, as pearled barley in treating spontaneous breast cancer in mice (15), barley warrants further study.

Corn and cornstarch are the chief sources of carbohydrate in the diet used for stock rats in this laboratory. Because of reproductive

failure and the high incidence of a lung congestion encountered in stock animals, this portion of the experiment was designed to study the influence of substituting barley for corn in a semi-synthetic ration.

Results

All animals grew well on the grain diets, had smooth, glossy coats, and were active throughout the experiment. The corn-fed animals were plumper and less active than the barley-fed rats. Barley-fed male rats made the least gain for each gram of food consumed, but it was noted that feces was more bulky on the barley diet and contained much visible fiber. It is possible that the laxative effect of the fiber prevented the ultimate utilization of the diet, since the caloric value of the diets was comparable. One male rat died after consuming the barley diet for 3 days, and routine autopsy disclosed an intestinal obstruction of fibrous material.

The male control rats gained the most weight per gram of available protein and corn rats the least.

From Table 7 it will be noted that barley-fed females produced more tissue per gram of food eaten and also per gram of protein than either those fed corn or the control rats.

The calculated nutrient values of the diets are comparable. The daily intake of essential amino acids was calculated from data compiled by Block and Bolling (1). Although the barley-fed rats, by consuming more diet, had a higher total protein intake, in the amounts

TABLE 7—*Weight gains and utilization of food by first-generation animals*

Sex	Group	Number of animals	Mean weight				
			At weaning	Gained in 200 days	Of young reared	Of tissue produced gm. food eaten	Of tissue produced gm. protein eaten
			gm.	gm.	gm.	gm.	gm.
Male	Control	4	61	423	.....	.113	.304
	Barley*	4	64	373	.....	.091	.369
	Corn	3	74	437	.....	.115	.271
Female	Control	4	56	195	237	.115	.515
	Barley	4	60	197	601	.170	.698
	Corn	4	59	200	214	.108	.562

*One died with an intestinal obstruction after 3 days on the diet.

TABLE 8—*Weight gains of second-litter, second-generation animals*

Group	Number of animals		Mean weight			
			At weaning		Gained in 150 days	
	Male	Female	Male	Female	Male	Female
			gm.	gm.	gm.	gm.
Control	11	5	50	47	310	344
Barley	14	13	51	48	273	163
Corn	9	3	70	54	348	193

eaten, the three diets furnished similar quantities of arginine, methionine, tryptophane and valine. The barley diet furnished less isoleucine, leucine, lysine and threonine than the control or corn diets and more histidine and phenylalanine. All three diets supplied the essential amino acids in excess of the amounts recommended by Griffith and Farris (9). Barley-fed females excelled in reproductive performance and it might be of interest to explore further the apparent superiority of the diet in this respect.

Autopsy revealed moderate fat deposits in the bodies of the control animals. The corn-fed rats were in excellent condition but had large accumulations of fat in the abdomen. The barley-fed animals had a minimal amount of fat, but had a well developed musculature.

Table 9 records the hemoglobin concentrations and red cell counts of these animals. There were no significant differences between groups. The mean erythrocyte counts for both male and female animals fed barley were slightly higher than those fed corn or control diets. All were superior to rats of the same strain at the same period in the life span as reported by Donaldson (3).

TABLE 9—*Hemoglobin determinations and erythrocyte counts for rats of the first-generation on the experimental diets*

Sex	Group	Hemoglobin gm./100 ml. of blood		Erythrocytes million/c.m.m. of blood	
		Mean	S.D.	Mean	S.D.
Male	Control II	15.7	.672	7.9	.625
	Barley	15.8	.501	8.22	.698
	Corn	15.6	.444	7.79	.476
Female	Control II	15.4	.70	8.62	.520
	Barley	15.7	.733	8.77	.450
	Corn	15.4	1.1	8.70	.955

TABLE 10—*Reproductive performance of two generations of rats*

Group	Generation	Litter number	Number of females		Young		
			Mated	Bearing litters	Total born	Percent reared	Mean weight at 28 days
							gm.
Control	1	1	4	3	27	11.1	51.3
	1	2	4	3	22	72.7	49.5
	2	1	4	3	17	70.5	45.4
Barley	1	1	4	4	27	75.8	48.4
	1	2	4	4	38	75.0	49.6
	2	1	13	7	56	61.0	48.3
Corn	1	1	4	4	32	3.1	65.0
	1	2	4	3	29	41.3	66.0
	2	1	3	2	14	21.4	52.0

The hemoglobin values and erythrocyte counts for all of these rats compare favorably with the results of studies made by Reich and Dunning (25) on rats of other strains. For the period of 28 to 200 days of age in over 2,000 rats, they found an average value of 13.6 gm. of hemoglobin per 100 ml. of blood, and 7.9 million erythrocytes for each cubic millimeter of blood. It is concluded that feeding barley and corn has little effect on blood formation.

Data for the first and second litters of the parent animals are presented in Table 10. More young were born to and reared by the barley-fed females than by females fed either of the other diets. Barley-fed females outranked stock females also in the number and percentage of young reared. Slonaker (29) reports a mean of 4.3 young born per female, and 60.3 percent of these young weaned at a comparable protein intake. Macomber (17) found that females fed 20.8 percent protein were able to rear 93 percent of their young.

The weight at weaning of young from barley-fed animals was comparable with stock young when the number reared is considered. Young from corn-fed animals attained more weight proportionately than any of the other young.

The sex ratio of the young is interesting. The ratio of males to females of barley young is 1: 1, corn 2: 1, and control 3: 1. As the sex of the newborn was not determined it is not known if this is a matter of survival. On the same protein intake, Slonaker (29) found a 1: 1 ratio to exist, and King (13) reports 107.3 males to 100 females. The latter author assumes that the animal in prime condition pro-

duces a litter of 1: 1 ratio or nearly that, and that an excess of male young are born to females either before or after their reproductive prime or when they are in poor physical condition.

Cannibalism caused the deaths of 70 percent of the total young born to corn-fed animals, 40 percent of the control young, and 18 percent of the young in the barley-fed group. Since the diets contained comparable amounts of protein, and since all but barley contained 14 percent or more of casein, cannibalism cannot be attributed to a lack of protein.

Two of the control females and one of the females fed corn failed to bear two litters. There was evidence of resorption in the corn fed and in one of the control females.

As with the parent animals, more young were born to, and reared by, the barley-fed young than by the second generation of either of the other dietary groups. The corn young attained the greatest weight, owing probably to the smaller litters.

The incidence of the chronic rat lung congestion was higher in the barley-fed animals than in either of the other groups. In both parents and young an equal number of males and females were affected, but at autopsy it was noted that females were more severely affected. This was true only of the barley-fed rats, and more males than females of the other two groups were affected.

TABLE 11—*Incidence of lung lesions in two generations of rats as revealed by gross autopsy*

Sex	Group	Lungs	
		Normal	Abnormal
		Number of rats	Number of rats
Male	Control II	10	6
	Barley	4	13
	Corn	7	2
	Stock	3	11
Female	Control II	7	1
	Barley	4	12
	Corn	7	0
	Stock	12	37

Summary—Barley and Corn

Neither barley nor corn affected the blood picture when these grains were the chief ingredient of rat dietaries.

Barley feeding resulted in a better reproductive performance than corn. A greater percentage of the young born were reared in both the first and the second generation.

Corn protected the rat from a chronic lung congestion to a greater extent than barley. The majority of corn-fed rats exhibited no lesions, while the majority of the barley-fed animals were affected.

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administer prophylactic treatment for tetanus and gas gangrene.

If called to treat a snake bite, as some of us occasionally are, act quickly. Lay the wound open and apply suction by some means constantly, with tourniquet applied. Do not hesitate to give morphine in large doses, and give the serum, repeating the dose in four hours or earlier if the patient lives. Give supportive treatment intravenously, such as glucose and saline. Remember that some lives may be saved. Out of three or four cases I have seen, I recall only one patient who lived.

In the smaller communities and rural districts, injuries resulting from automobile accidents frequently exact all our skill and ingenuity. For instance, I was recently called to treat a case of simple fracture of the tibia and fibula and a compound fracture of the femur on the left side in a patient suffering from shock and loss of blood. This condition was complicated by a fractured pelvis with involvement of the hip joint, also on the left side, which was neither complained of nor recognized until some time after fixation of the fractured femur had been completed and other emergency treatment given.

CONCLUSION

Emergency procedures in general practice tax the ingenuity and skill of the physician. They are sufficiently difficult with the most modern conveniences of the up-to-date hospital, but in a community without these facilities, the physician's responsibility is greatly increased. I have outlined only a few of the conditions that have come under my observation.

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GARLIC: AN OCCUPATIONAL FACTOR IN THE ETIOLOGY OF BRONCHIAL ASTHMA

CASE REPORT

GRAHAM E. HENSON, M. D.
Jacksonville

A case of more than usual interest is that of A. A., an adult white male who presented himself with the following history.

A former resident of Minnesota, 22 years of age, married, he had suffered with a seasonal pollinosis for four years. In 1937 he had been given cutaneous tests for all of the tree, grass and weed pollens indigenous to the section of the country in which he was living and had been found very definitely positive to the entire ragweed group. He was given the common ragweed pollen extract subcutaneously in the customary increasing doses reaching a maximum dosage of 6,000 units, acquiring a tolerance that rendered him free of symptoms for the balance of that season and the greater part of the 1938 season.

Late in September of 1938 he developed a return of his "hay fever" symptoms but in a less aggravated form than in previous attacks. Just a month later he developed a bronchial asthma of a mild nature with attacks occurring every evening, increasing in both duration and intensity so that when he first came under my observation, on November 11, he was losing four or five hours of sleep each night. A complete allergic study was undertaken, giving all the food protein, epidermal and miscellaneous protein, the tree, grass and weed pollen extracts and the bacterins. Both the cutaneous and intradermal methods were employed. His tolerance to the ragweed group had become lost as evidenced by positive reactions to the entire group—lineal cutaneous tests of 1 cm., producing urticarial wheals from 3 to 4 cm., at their greatest diameters. He gave negative reactions to all the epidermal, miscellaneous and bacterial protein extracts and to all the food proteins with the exception of garlic. A lineal cutaneous test of this vegetable protein extract measuring 1 cm., produced an urticarial wheal 4 cm., at its greatest diameter with numerous pseudopodia measuring from 1 to 2 centimeters.

His occupation was that of a foreman in the sausage department of a large wholesale meat packing plant. In the making of sausage a powdered garlic was being used and the air permeated with the odor of garlic at all times. At my suggestion he substituted the garlic kernel for the powdered form. On the evening of the second day following this substitution he went to bed at an early hour, slept all night and has been entirely free of any symptoms of asthma since that time, now a period of over seventeen months.

The nightly occurrence of asthmatic attacks increasing in intensity, the very positive reaction he gave to garlic, together with the remarkably prompt disappearance of all asthmatic symptoms as soon as the offending powder was removed, leave no doubt that that part of his occupation involving the use of powdered garlic was the sole etiological factor of his asthma.

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CLASSIC ASPECTS OF ONION AND GARLIC DERMATITIS IN HOUSEWIVES

J. W. BURKS, JR., M.D.
New Orleans, Louisiana

CONTACT dermatitis of the hands in housewives constitutes a common and difficult diagnostic problem. Its frequency has been augmented in recent years by the increasing variety of objects coming in contact with the hands, including the newer plastic materials and more efficient detergents. Recognition of certain distinctive anatomic patterns facilitates early diagnosis. The value of such landmarks has been emphasized in the monographs of Waldbott[16] and Ronchese[11], who have compiled lists of the more common patterns. The present report concerns an additional distinctive pattern which I have observed and studied during the past ten years in the fingers of housewives as a result of contact with onions or garlic.

Onions and garlic are members of the *Liliaceae*, a family closely related to the *Amaryllidaceae*, which includes narcissus, daffodil, campernelle, jonquil, polyanthus, and chinese sacred lily, and to the *Iridaceae*, which embraces iris and gladiolus. Although plants of the lily family are not common irritants, they are known sensitizers. Derbes[3] reported a case of hay fever and asthma caused by narcissus bulbs, and Henson[7] described another in which powdered garlic was the responsible agent. "Tulip fingers," according to Bertwistle[1], occur in 85 per cent of the workers in Holland. "Lily rash" is common in the Scilly islands, where vast numbers of narcissi are grown. In the United States, as well as in Europe, dermatitis has been traced to hyacinths, and Stryker[12] found jonquils to produce dermatitis in flower growers of this country. Garlic has been known to cause the phenomenon of cutaneous sensitivity either by its bulb or by its pollen.[13] In a case reported by Burgess,[4] dermatitis of the fingers and palm of the left hand in a cook resulted from paring onions and garlic. Edelstein[5] described dermatitis of the wrist in a meat grinder after he had rubbed this area against debris containing garlic.

The following description of a case, the first observed by me, is typical of this syndrome.

REPORT OF A CASE

F. M., a thirty-seven-year-old white woman stenographer, had an eruption of the tips of the thumb, index, and middle fingers of the left hand as well as the tip of thumb of the right hand. (Fig. 1). Exacerbations and remissions had alternated continuously for several years. There was no family history of allergic diseases.

Dr. Burks is Assistant Professor of Medicine (Dermatology), Tulane University Medical School, New Orleans, Louisiana.

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Invariably, the eruption was worse on Monday mornings and actually began to flare up on Saturday nights. Detailed questioning regarding possible contact agents in her office and home yielded no pertinent information. She could think of nothing she touched with these three fingers of her left hand that did not come in contact

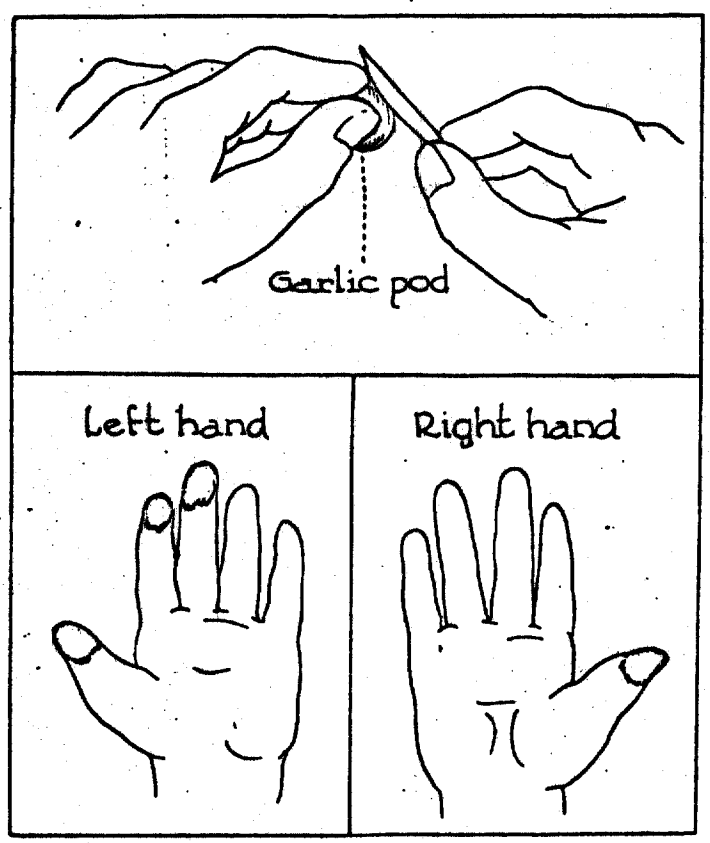

Fig. 1. Characteristic distribution of onion and garlic dermatitis of the hands of housewives. Note eruption on the tips of the thumb, index, and middle fingers of one hand and the tip of the thumb of the other.

with her right hand, for she was right-handed. Further questioning revealed that she was fond of cooking but, because of the nature of her occupation, dined in restaurants on weekdays. On Saturdays, she prepared her own meal, usually roast beef, which she seasoned with chopped onions and garlic. These she held with her left thumb, index and middle fingers while she chopped with a knife held in the right hand, the lateral radial aspect of the right thumb hitting the chopping board with each stroke.

Patch tests were made by applying cut, raw surfaces of onions and garlic to the volar aspect of the left forearm near the wrist. Within twenty-four hours a severely pruritic vesicular dermatitis appeared. Reaction to garlic was more severe and was prolonged to three weeks. Areas of dermatitis of the fingers flared during patch testing.

Avoidance of further contact with onions and garlic through use of protective

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creams and gloves, along with soothing local treatment of the dermatitis, effected complete remission. Three months later, she had another attack after planting lily bulbs. Patch test to the cut, raw surface of this bulb yielded a positive reaction. On another occasion she attributed a flare up to ingestion of garlic in a salad, but no intradermal testing was done.

COMMENT

Since this initial observation, numerous similar cases have been recognized. In one patient, a housewife, the reversal of localization to the left thumb and the right thumb, index and middle fingers was due to the fact that she was left-handed. In another instance, the left-handedness of a patient was accurately predicted from these clues.

Because onions and garlic are used so extensively for seasoning in Louisiana, as in other southern states, the incidence of this type of dermatitis is much higher in these areas. It is common practice for the housewife to hold the bulb of these plants against a chopping board with the thumb, middle and index fingers and to hold the knife in the opposite hand, the blade being grasped by all fingers and the handle extending across and beyond the palm. As she chops, the lateral radial aspect of the thumb hits the chopping board and the chopped substance. Another technique consists in holding the pod between the thumb, index and middle fingers of one hand and paring against the thumb. When large onions are used, the contact pattern follows a distribution which coincides with the points of contact made by holding a ball in the hand. Waldbott[16] has classified this localization as a "finger grip type" of pattern, arising from gripping such articles as knobs, office equipment, kitchen drawers, metal or plastic bottoms, small pieces of jewelry, lipstick containers, pills, tablets, popcorn, peanuts, and olives. In these instances, however, three digits of the right hand and one of the left are usually involved because of the predominance of right-handedness, whereas the onion and garlic pattern commonly occurs on the three digits of the left hand, which hold the plants.

PATCH TESTING

Earlier, Edelstein[8], Burgess[4], and others applied the cut, raw surfaces of onions and garlic to the unbroken skin of the wrist of patients with this pattern and obtained positive reactions, the severity in some cases suggesting the action of a primary irritant. Several years ago I used the irritant action of onion to produce vesiculation for treatment of "creeping eruption." In one such patient, patch testing to onions three weeks after cessation of treatment with onion poultice yielded a negative reaction. Apparently, the irritant action varies with the potency of certain varieties of the plant.

Extracts were prepared in an effort to eliminate the possibility of primary irritation and to discover which fraction (oil or water soluble) was the offending allergen. Fifteen grams each of onion and garlic were

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TABLE I. RESULTS OF PATCH TESTING WITH ONIONS AND GARLIC

Type of Dermatitis	Water Soluble Fraction Reactions				Fat Soluble Fraction Reactions				Total Number Subjects
	Onions		Garlic		Onions		Garlic		
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	
Onion and/or garlic dermatitis	6	4	2	8	9	1?	9	1	10
Unclassified eczematoid dermatitis of hands	3	2	3	2	5	0	5	0	0
Control	10	0	10	0	10	0	10	0	10

chopped separately and macerated with mortar and pestle and were placed in separate filters. Five ounces of ether in one case and five ounces of water in the other were poured over each of these. This filtrate was used in full strength for testing. The volar surface of the left wrist was cleansed with soap and water. An elastoplast type of patch with a central protective area of cellophane was applied over a bit of cotton saturated with the extract. Twenty-four hours later, patches were removed, except in a few cases in which almost immediate reaction took place. Readings were made at twenty-four, forty-eight, and seventy-two hours, and reactions were graded from one plus to four plus. One four plus reaction persisted as a localized dermatitis for three weeks. Of the last ten patients tested, one had a one plus reaction to the fat soluble extract of garlic and a questionably positive reaction to that of onion. Eight exhibited from two to four plus reactions to the aqueous extract of garlic, four of whom also had positive reactions to aqueous extract of onion (Table I).

An equal number of control subjects with no history of dermatitis was tested, all of whom had negative responses. Of five patients with unclassified eczematoid dermatitis of the hands, two demonstrated two plus reactions to the aqueous extract. Both of these had complained of burning of the hands after contact with onions and garlic. From these results it is apparent that the allergenic substance is water soluble.

DISCUSSION

The foreign literature contains contradictory reports regarding the actual mechanism of dermatitis produced by the lily family. Touton[14] incriminated a parasite in the cloves of garlic, but Overton[10] exonerated *Leptus autumnalis* as a cause for lily dermatitis. Earlier, in cases of hyacinth and tulip dermatitis, the traumatizing action of sharp-edged crystals of oxalate of lime was suspected and was found to be eliminated by treatment of the plants with weak hydrochloric acid. Hoffman[8] considered the sensitizing agent to be not a glucoside but an etheral oil. Touton,[14] Broers,[2] and Freeman[6] believed this oil to exist only in the juice of fresh bulbs of the tulip, whereas Kranenburg[9] and Waanders[15] found the offending substance in dry hyacinths. Touraine and Neret's[13] patient was sensitive both to the raw fresh bulbs and to extracts, and, in their

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article on occupational tulip dermatitis, they concluded that the theory of parasites and crystals must be discarded.

The odor of onions and garlic has been found to be due to essential oils, in onions mainly allylpropyl disulphide, which also appears in small amounts in garlic along with the main constituent of diallyl disulphide. In addition, these plants contain other sulphur-bearing oils of unknown chemical composition. The coexistence of positive reactions to onions and garlic would tend to suggest an offending oil common to both.

SUMMARY

A hitherto unreported distinctive pattern of contact dermatitis of the hands of housewives due to onion and garlic is described, involving the thumb, index and middle fingers of one hand and the thumb of the other. Studies with raw and extracted substances of these plants indicate an allergenic as well as a primary irritant action. Contrary to previous reports, the allergenic extract was found to be water soluble rather than fat soluble.

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THE MODUS OPERANDI OF CARMINATIVES

The Therapeutic Value of Garlic in Functional Gastrointestinal Disorders

FREDERIC DAMRAU, M.D.
and
EDGAR A. FERGUSON, Chemist
New York, N. Y.

The value of garlic as a carminative has been recognized since ancient times. A Sanskrit document, the Bower manuscript found by a native in the ruins of Mingai, Turkestan, contains a remarkable dithyramb in praise of *allium sativum* (garlic)[1]. In the rich materia medica of India, Súsruta in the fifth century A. D. described a number of carminatives which are still in use today. Hippocrates, Pliny, and Galen used garlic widely in therapeutics.

It is believed that the clinical and roentgenographic investigations reported in this paper throw a new light on an ancient subject. In a series of cases of functional dyspepsias and gastric neuroses treated with dehydrated garlic tablets, we observed definite and consistent relief of the symptoms of heaviness after eating, flatulence, gas colic, belching and nausea.

Of special interest in these cases was the associated reduction or disappearance of gas accumulations in the stomach and intestines as revealed by the roentgenograms. In other words, the before and after roentgenographic comparisons confirmed the clinical improvement.

Very few scientific studies on the *modus operandi* of carminatives have been published and their action has largely been taken for granted. Carminatives have been defined by Sollmann[2] as "aromatic and pungent drugs, used in flatulence and colic, to expel gas from the stomach and intestines, and to diminish the 'griping' pains".

The action of carminative oils on the alimentary canal is explained by Cushny[3] as follows: "On passing into the stomach, the oils cause the same sensation of warmth in the gullet, and this is accompanied by a sense of well-being and comfort, the appetite is often increased, and any feeling of distention after meals is relieved. This is often attended by the eructation of quantities of gas. Substances which produce these effects in the stomach are known as *carminatives*, and many explanations of their action have been offered . . . The movements and tone of the stomach are decreased by small quantities of the oils applied to the mucous membrane; this weakening action probably extends to the sphincters, and their relaxation may explain the relief of the feeling of distention and the eructation of gas from the stomach after the administration of these oils".

We believe that our studies have confirmed Cushny's theory that carminative oils reduce the movements and tension of the stomach and also relax the sphincters, thus relieving flatulence and its attending symptoms. So far as garlic is

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concerned, our investigation proves that this pharmacodynamic action extends along the intestinal tract.

In view of the fact that so little has been published on the uses of garlic in gastrointestinal therapy, although its carminative action has been taken for granted for centuries, we believe that a report of our original studies will be of special interest in the field of gastroenterology.

Gastroenteric Allichalone

What is the nature of the carminative action of dehydrated garlic? As has been stated already, this was evidenced clinically by symptomatic relief of flatulent dyspepsia. An unexpected finding of our research, as disclosed by the gastrointestinal roentgenograms, was the discovery that dehydrated garlic exerts a delaying action upon excessive motor activity of the stomach and intestines. Garlic retards hyperperistalsis.

It is apparent from our studies that there are unidentified principles in garlic which exert a sedative action upon the plexuses of Meissner in the submucosa of the stomach and small intestines and perhaps also upon the plexuses of Auerbach in the muscular coats of the intestines. We believe that garlic relaxes the tone and motility of the stomach and intestines by acting on the nerve endings in the mucous membrane. Much of the clinical improvement following administration of dehydrated garlic in gastric neuroses and flatulent dyspepsia is, in our opinion, due to this *chalonic* (relaxing) action.

To these unidentified principles in garlic, in view of their unusual pharmacodynamic action, we have given the descriptive name *gastroenteric allichalone* (*allium*, garlic; *chalone*, to relax).

Roentgenographic Study

A preliminary roentgenographic study was undertaken to determine the effect of dehydrated garlic on gastrointestinal motility by comparison of the opaque residues with and without the garlic. For this purpose standard barium sulfate meals were administered according to the customary method for a gastrointestinal series. The comparison roentgenograms were taken six hours after ingestion of the barium.

The series studied consisted of 25 patients referred to a gastroenterological clinic. Twelve were males and thirteen females. The average age was 42, height 5 feet 5 inches, weight 142 lbs. The average duration of the complaint was one year. Eighteen of the patients complained of abdominal pain, 8 of epigastric or abdominal discomfort, 8 of belching and 11 of nausea.

In all cases the roentgenographic comparison was made on the same patient with and without the garlic. In the odd number cases, the first test was made with the garlic tablets and repeated ten days later without medication. In the even number cases, the first test was made without medication and repeated ten days later with garlic tablets.

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The medication used consisted of a total of 6 garlic tablets, each containing 4¾ grains of dehydrated garlic. Two tablets were given with the barium meal, 2 two hours later, and the remaining 2 tablets after four hours.

With the use of garlic the size of the barium residue in the stomach after six hours was definitely larger than without the medication. In the horizontal axis, the measurement of the barium residue in the stomach averaged 1.7 cm. with medication as compared with 0.76 cm. without medication. In the vertical axis, the barium residue averaged 0.88 cm. with medication as compared with 0.16 cm. without medication. With the use of the dried garlic tablets part of the barium meal was retained in the stomach after six hours in 7 cases as compared with 3 cases without medication. This change in the size of the barium residue demonstrates a sedative action of *allium sativum* on gastric motility.

To determine the delaying action of garlic in the intestines, the station position of the head of the barium column was measured as follows: still in the stomach, 1; duodenum, 2; small intestines, 3; ileocecal junction, 4; hepatic flexure, 5; center of transverse colon, 6; splenic flexure, 7; sigmoid, 8; rectum, 9; complete evacuation, 10.

Without medication the average station in the series reached by the head of the barium meal was 6.0. With administration of garlic, the progress of the residue was reduced to an average of 5.7 stations. Considering the fact that a total of only 28 ½ grains of dehydrated garlic was administered and the gastrointestinal tract is 30 feet long, an average reduction of 0.3 station represents a definite delaying action on intestinal peristalsis. Roughly it may be assumed to be approximately an average of 10 inches.

In this limited study we were impressed by the patients' reports of relief of flatulence, belching and gas colic when the garlic tablets were taken. Hence we decided to give the garlic a more extensive trial and administer it over a sufficient period of time to provide a reasonable opportunity for therapeutic benefit.

Clinical Studies

In a series of 29 patients complaining of heaviness after eating, belching, flatulence, gas colic and nausea, 2 garlic tablets were given twice daily, after lunch and dinner, for a period of two weeks. Each tablet contained 4¾ grains of dehydrated garlic per tablet, flavor modified with 2⅜ grains of dehydrated parsley. The tablets were sugar coated for palatability and dissolved readily in the stomach.

The clinical group consisted of 15 females and 14 males. The average age was 43, height 5 feet 5 inches, weight 152 lb. The average duration of the complaint was 1.2 years.

For the purpose of determining the degree of benefit after medication, the symptoms were numerically rated as to severity as follows:

Heaviness after eating, a form of epigastric or abdominal distress without colic pain, is generally regarded as consciousness of peristaltic action. It was

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rated as *one plus* when present, *two plus* when it interfered with sleep, and *zero* when absent.

Belching was rated as *one plus* when it was sufficient to cause temporary disturbance following a meal, *two plus* when the discomfort continued and interfered with sleep, *three plus* when it was necessary to get up during the night and take special measures during the day to relieve continuous belching, and *zero* when absent.

Flatulence was rated as *one plus* when it was easily relieved by belching, *two plus* when there was some distention of the stomach, *three plus* when the distention was obvious and could not be relieved, and *zero* when absent.

Colic, or spasmodic abdominal pain due to gas, was rated as *one plus* when present, *two plus* when severe enough to interfere with work and sleep, and *zero* when absent.

Nausea was rated as *one plus* when present, *two plus* when it interfered with eating, *three plus* when it caused vomiting, and *zero* when absent.

On the basis of these ratings, the severity of the symptoms was averaged for the series of 29 cases before and after treatment with dehydrated garlic tablets for a period of two weeks. The results are summarized in the table.

TABLE I

RELIEF OF SYMPTOMS BY GARLIC TABLETS
Average Severity before and after Medication

Symptom	*Before*	*After*	*% Relief*
Heaviness	1.4	0.44	69%
Belching	1.7	0.48	72%
Flatulence	1.2	0.2	83%
Gas Colic	1.5	0.5	67%
Nausea	1.1	0.5	55%

An analysis on the basis of cases gives the following results:

Heaviness after eating (epigastric or abdominal distress) was present in 25 cases. It was completely relieved by the treatment in 15 cases, partially relieved in 6, no relief in 4 cases.

Belching was present in 25 cases. It was completely relieved by the treatment in 13 cases, partial relief in 9, no relief in 3 cases.

Flatulence was present in 25 cases. It was completely relieved by the treatment in 20 cases, partial relief in 1, no relief in 4 cases.

Gas colic was present in 24 cases. It was completely relieved by the treatment in 13 cases, partial relief in 8, no relief in 3 cases.

Nausea was present in 8 cases. It was completely relieved by the treatment in 6 cases, no relief in 2 cases.

The statistics given in the above table and analysis show significant relief of the common symptoms of flatulent dyspepsia, nervous dyspepsia and other gastric neuroses and suggest the use of dehydrated garlic tablets for relief of a wide variety of gastric and intestinal symptoms.

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In this second series of cases, when studied roentgenographically, it was likewise found that the gastric residue was increased and the progress of the head of the barium meal delayed after two weeks of medication with dehydrated garlic tablets.

Case Reports

The following case reports are representative of therapeutic results with complete symptomatic relief obtained with dehydrated garlic tablets in this series of cases.

Case 1:—E. C., age 55, a married woman with two children, height 5 feet 4 inches, weight 162 lb., complained of heaviness after eating, flatulence, gas colic, belching, nausea, nervousness and loss of appetite. The duration of these symptoms was six months. No demonstrable lesions were found on clinical and roentgenographic study.

After taking dehydrated garlic tablets for two weeks, 2 tablets twice daily after lunch and dinner, the symptoms of heaviness, gas colic and nausea were completely relieved. There was considerable relief of flatulence and belching. She was less nervous and her appetite improved.

Because roentgenograms of this case are typical of the series, they are reproduced herewith.

Figure 1, before medication, shows a very large stomach and redundant bowel, extending up to the splenic flexure then down to a wide, enlarged sigmoid. A large amount of gas may be observed at the splenic flexure. Accompanying clinical complaints, as in this case, are usually flatulence or gas in the stomach, which is ordinarily not relieved by belching. Six hour study (Figure 2) shows barium in the sigmoid and partial evacuation. Evacuation is complete in twenty-four hours (Figure 3).

The entire process is slowed after medication (Figures 4, 5, 6). In the six hour plate (Figure 5), the head of the barium column is in the descending colon and does not extend into the sigmoid. The twenty-four hour plate (Figure 6) shows partial evacuation and the sigmoid is not distended with gas. This is an extremely beneficial effect of garlic medication and typical of the entire series.

Case 2:—A. F., age 50, a married woman with one child, height 5 feet 3 inches, weight 180 lb., complained of epigastric distress (heaviness after eating), belching, flatulence and gas colic. The duration of the complaint was one year. No demonstrable lesions were found on clinical and roentgenographic study.

The medication prescribed was 2 dehydrated garlic tablets twice daily, after lunch and dinner. At the end of two weeks on this treatment, there was no longer any distress and all symptoms of heaviness, belching, flatulence and gas colic had disappeared.

Before medication there was complete evacuation in twenty-four hours and the roentgenogram showed no residual barium. After medication with garlic the

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twenty-four hour film showed a great deal of residuum, resulting from the slowing action on intestinal motility. Another interesting feature was that, after administration of garlic, most of the gas had moved along the intestine with the barium instead of remaining in the stomach as shown in the previous film.

This is a case of flatulent dyspepsia plus hyperperistalsis of the lower bowel, relieved symptomatically and objectively in two weeks by garlic medication.

Case 3:—K. L., age 19, male, single, height 5 feet 10 inches, weight 160 lb., complained of continuous belching which was so severe as to interfere with sleep. Further complaints were flatulence, epigastric distress (heaviness after eating), gas colic and anorexia. The duration of the illness was one year. No demonstrable lesions were found on clinical and roentgenographic study.

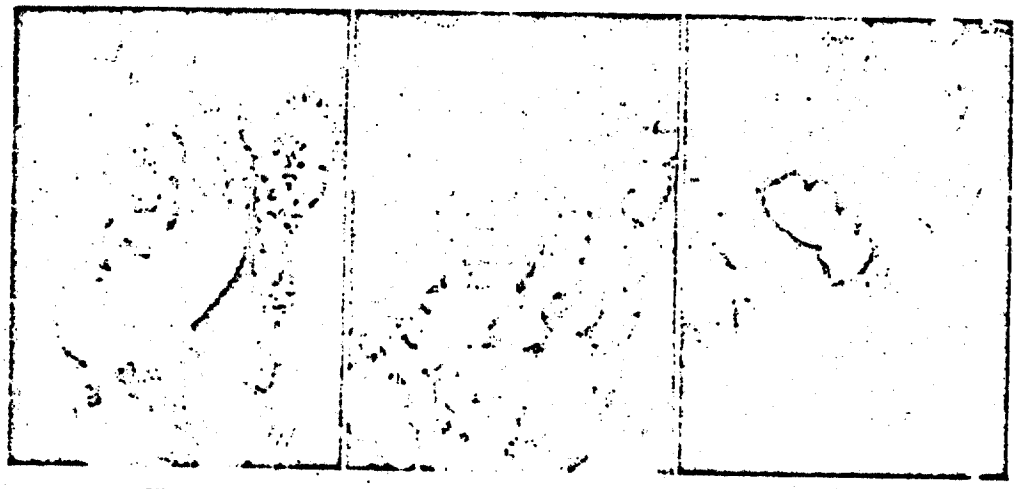

Fig. 1 Fig. 2 Fig. 3

Fig. 1—Case 1. Before medication. One-half hour after barium meal.
Fig. 2—Six hours after barium meal.
Fig. 3—Twenty-four hours after barium meal.

The patient was put on the prescribed routine of 2 dehydrated garlic tablets twice daily, after lunch and dinner. At the end of two weeks all of the symptoms were completely relieved and the appetite had improved.

A comparison of the stomach roentgenograms taken one-half hour after the barium meal showed extreme hyperperistalsis originating in the stomach before medication, which slowed down considerably after medication. At six hours the head of the barium column, before medication, reached the sigmoid and approached the rectum. After medication, it reached only as far as the middle of the transverse colon and most of the residual barium remained in the ascending colon, demonstrating a great deal of retardation.

In this case the symptoms of hyperperistalsis originating in the stomach were relieved completely by garlic medication and comparison of the before and after roentgenograms proved that the *modus operandi* was by means of the *gastro-enteric allichalone* action.

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Case 4:—E. E., age 52, a married woman with two children, height 5 feet 3 inches, weight 120 lb., complained of intractable belching which kept her awake nights, flatulence, gas colic, epigastric distress (heaviness after eating), loss of appetite and nervousness. Her symptoms dated back three years. No demonstrable lesions were found on clinical and roentgenographic study.

After two weeks of treatment with dehydrated garlic, 2 tablets twice daily after lunch and dinner, the symptoms of belching, flatulence, gas colic and epigastric distress were completely relieved. Appetite has improved and she was less nervous.

Before medication, the six-hour film showed partial evacuation of the barium meal resulting from hyperperistalsis. After medication, the head of the barium

Fig. 4 Fig. 5 Fig. 6

Fig. 4—Case 1. After 2 weeks medication with dehydrated garlic tablets. One-half hour after barium meal.
Fig. 5—Six hours after barium meal.
Fig. 6—Twenty-four hours after barium meal.

column reached only as far as the middle of the transverse colon. Hence the treatment not only relieved the symptoms of flatulent dyspepsia but also checked the excessively rapid peristaltic action.

Case 5:—M. S., age 42, a married woman with three children, height 5 feet 3 inches, weight 150 lb., complained of flatulence and of continuous belching day and night. Her symptoms had lasted one year. No demonstrable lesions were found on clinical and roentgenographic study.

There was complete relief of these symptoms after two weeks of medication with dehydrated garlic, 2 tablets twice daily after lunch and dinner.

Roentgenographic study of the half-hour stomach films before use of garlic tablets showed gastric hyperactivity. The stomach contents moved immediately

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on through the duodenum into the small intestine. This was not evident after medication. The twenty-four hour film before medication showed the head of the barium column in the rectum. After medication it reached only as far as the splenic flexure.

Case 6:—S. W., age 58, a married woman with one child, height 5 feet 2 inches, weight 152 lb., complained of belching for the past two years, which was so severe that she had to get up at night to take special measures for relief as well as during daytime. Flatulence was so severe that distention of the stomach and intestines was obvious and could not be relieved by ordinary means. No demonstrable lesions were found on clinical and roentgenographic study.

Relief of both belching and flatulence was complete after the use of dehydrated garlic tablets, 2 tablets twice daily after lunch and dinner, for a period of two weeks.

There was excessive hyperperistalsis, as shown by the roentgenograms. The six-hour film without medication showed the head of the barium meal in the rectum with scattered barium throughout the colon. After garlic medication, part of the barium remained in the small intestines and the rest was visible from the cecum past the hepatic flexure to the transverse colon. The treatment definitely reduced the hyperperistalsis.

Case 7:—C. C., age 42, a married man, height 5 feet 7 inches, weight 163 lb., complained for the past seven months of very severe belching, flatulence and gas colic, together with epigastric distress (heaviness after eating). He had lost weight and was nervous and unhappy. No demonstrable lesions were found on clinical and roentgenographic study.

After taking dehydrated garlic tablets for two weeks, 2 tablets twice daily after lunch and dinner, the symptoms of belching, flatulence, gas colic and epigastric distress were completely relieved. He gained a pound and was less nervous and depressed.

Twenty-four hour films before and after medication showed delayed evacuation. The head of the barium column reached the descending colon and sigmoid. It is interesting to note that garlic did not increase the stasis in this type of case.

Case 8:—W. W., age 50, a married man, height 5 feet 8 inches, weight 156 lb., complained for the last eight months of severe abdominal distress (heaviness in the hypogastric region after eating), and also of belching and flatulence. He was very nervous. No demonstrable lesions were found on clinical and roentgenographic study.

He experienced complete relief of the abdominal distress, belching and flatulence after taking the dehydrated garlic tablets as directed for two weeks. He was less nervous and there was no longer any area of abdominal distress.

At six hours the roentgenograms both before and after medication showed complete emptying of the stomach and the head of the barium meal had reached the middle of the transverse colon.

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Summary

A clinical investigation of dehydrated garlic showed this carminative to be highly effective for relief of heaviness after eating (epigastric and abdominal distress), belching, flatulence, gas colic, and nausea. Satisfactory therapeutic results were obtained in cases of flatulent dyspepsia, nervous dyspepsia and other gastric neuroses.

Roentgenographically a comparison of films with and without the medication demonstrated that dehydrated garlic has a sedative action on the stomach and intestines, relaxes spasm, retards hyperperistalsis and disperses accumulations of gas.

It is believed that these studies explain the carminative action of garlic as due to unidentified principles which we have designated as *gastroenteric allichalone* (*allium*, garlic; *chalone*, to relax).

Since dehydrated garlic tablets are safe for long continued use, they may be indicated in a wide variety of functional disturbances of the stomach and intestines.

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Enzyme Inhibition by Allicin, the Active Principle of Garlic

By E. D. WILLS
Department of Biochemistry and Chemistry, Medical College of St Bartholomew's Hospital, London, E.C. 1

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Extracts of garlic (*Allium sativum*) have been used for medicinal purposes for several thousand years. Their use is described by Virgil in the *Second Idyll* as a treatment for snake bite and by Hippocrates for treating pneumonia and suppurating wounds. The use of garlic extracts for treating such ailments as gastric catarrh, dysentery, typhoid and cholera has continued in many parts of the World. Recently garlic extracts have been claimed to be more satisfactory than penicillin for the treatment of some throat infections (Fortunatov, 1952).

A scientific basis for the medicinal use of garlic extracts was established by Lehmann (1930), who demonstrated that the growth of *Eberthella typhosa* and *Escherichia coli* was abolished by the extracts. The true nature of the active principle, however, remained obscure until Cavallito & Bailey (1944), by steam distillation of ethanolic garlic extracts, obtained a colourless strong-smelling oil which possessed a powerful bactericidal action in concentrations as low as 1:85000, against a wide range of bacteria. This oil, called allicin, was diallylthiosulphinic acid (II) (Cavallito, Buck & Suter, 1944).

Stoll & Seebeck (1948), however, showed that the undamaged garlic bulb contained, not allicin, but a related substance, alliin, which was converted into allicin enzymically when the bulb was crushed. Alliin has no bactericidal action. Stoll & Seebeck (1951) showed by synthesis that alliin was *S*-allyl-L-cysteine *S*-oxide (I). Stoll & Seebeck (1949*a*) formulated its conversion into allicin as

$$\underset{\text{(I)}}{2R.SO.CH_2.CH(NH_2).CO_2H} \xrightarrow[\text{Alliinase}]{+H_2O} \underset{\text{(II)}}{R.SO.S.R} + 2CH_2.CO.CO_2H + 2NH_3$$

$$(R = -CH_2.CH{:}CH_2)$$

Garlic extracts inhibit alkaline phosphatase and invertase (Danilenko & Epshtein, 1953), urease and succinic dehydrogenase (Szymona, 1952), and blood peroxidase (Vinokurow, Bronz & Korsak, 1947), but do not affect carboxylase and cytochrome oxidase (Szymona, 1952), or amylase and pepsin (Vinokurow *et al.* 1947). Invertase activity of red-clover extracts is, however, stated to be increased by garlic extracts (Morozow, 1950). Allicin inhibits urease (Agarwala, Murti & Shrivastava, 1952), papain and amylase (Rao, Rao & Venkataraman, 1946). No study of the effect of alliin on enzymes appears to have been made.

The object of the present work has been, by the use of pure synthetic alliin, to establish whether enzyme inhibition observed with garlic extracts can be attributed to alliin or to allicin and to examine the possibility that the antibacterial action of allicin could be ascribed to enzyme inhibition. Twenty-eight enzymes have been tested, in the presence of various concentrations of alliin and allicin. The effect on a few enzymes of some substances with a structure related to allicin has also been examined, in an attempt to establish the mechanism of action of allicin.

EXPERIMENTAL

Materials

DL-*Alliin*. This synthetic product was a gift from Sandoz Ltd.

Allicin. This was prepared enzymically by incubating at 37° for 15 min. an alliin solution of the required strength with an equal volume of alliinase solution (see below). Allicin was synthesized as described by Small, Bailey & Cavallito (1947).

Diallyl sulphide (L. Light and Co. Ltd.). This was redistilled *in vacuo* before use.

Diallyl disulphide. This was synthesized from allyl chloride as described by Twiss (1914). It was distilled at 76° at 11 mm.

Diallyl sulphoxide. This was synthesized as described by Stoll & Seebeck (1948).

Enzyme preparations and activity measurements

The methods of preparation and estimation of activity for most of the enzymes used have been previously described (Wills & Wormall, 1950; Wills, 1954).

Alliinase. This enzyme was prepared by the method of Stoll & Seebeck (1949*a*) with the modification that the ammonia used for neutralization was replaced by NaOH since traces of ammonia interfere with the estimation of the enzyme activity. The enzyme extracted from 25 g. of garlic was finally made up in 25 ml. of 0·1M phosphate buffer, pH 6·0.

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The ammonia produced by alliinase action was estimated in a Conway vessel with 0·25 M phosphate buffer, pH 6·0 (0·5 ml.), 0·02 M alliin solution (0·5 ml.), water (0·5 ml.) and alliinase solution (0·5 ml.) in the outer compartment and 1% (w/v) boric acid containing mixed indicator (1 ml.) in the inner compartment. The vessel was incubated at 37° for 15 min., when a saturated solution of K_2CO_3 (1 ml.) was run into the outer compartment. The vessel was incubated for a further 60 min. at 37° and the boric acid finally titrated with 0·0059 N-HCl.

Succinic dehydrogenase. This was estimated as previously described (Wills & Wormall, 1950) and also, with a 10% (w/v) homogenate of rat liver, by the colorimetric method of Kun & Abood (1949).

Papain. This was estimated by a milk-clotting method already described (Wills & Wormall, 1950), and as previously described for trypsin (Wills, 1954).

Xanthine oxidase. (*a*) *From milk.* Preparations used were untreated cream, a cream extract in phosphate buffer described by Horecker & Heppel (1949) and samples of stages in the preparation of crystalline xanthine oxidase, which were kindly supplied by Professor F. Bergel. These were samples M1, M2, M5 and M7 (crystalline enzyme) as described by Avis, Bergel & Bray (1955). (*b*) *From liver.* This was prepared from rat liver either as described by Axelrod & Elvehjem (1941), or by I. Lewin (private communication). The enzyme activity was estimated manometrically with hypoxanthine as substrate, as previously described (Wills & Wormall, 1950).

Triose phosphate dehydrogenase. The enzyme source and method of estimation were as described by Mackworth (1948).

Lactic dehydrogenase. A 5% (w/v) aqueous extract of acetone-dried powder of rabbit muscle was used. Enzyme activity was measured by the Thunberg method with 0·2 M phosphate buffer, pH 7·4 (0·5 ml.), water (0·5 ml.), 0·1% (w/v) coenzyme I (Co I) solution (0·5 ml.) and enzyme solution (0·5 ml.) in the main tube, and 0·005% (w/v) methylene blue (0·2 ml.) and 2% (w/v) sodium lactate (0·2 ml.) in the side bulb. The tube was evacuated, warmed to 37° and the contents were mixed. The time for decolorization of the methylene blue was observed.

Alcohol dehydrogenase. (*a*) *From yeast.* This was prepared from dried yeast by the first stages of the method described by Racker (1950). (*b*) *From liver.* This was prepared as described by Lutwak-Mann (1938). The Thunberg method was used for the estimation.

Alkaline phosphatase. Ox or rabbit serum was used as a source of this enzyme. It was estimated by the method of King (1951).

Adenosine triphosphatase. An extract of rat skeletal muscle or cardiac muscle was used and its activity was estimated by the method of Dubois & Potter (1943).

RESULTS

Alliinase action and allicin production

It has been previously shown that natural L-alliin is completely and rapidly converted into allicin, ammonia and pyruvic acid by alliinase. The enzyme was, however, optically specific and did not attack the D-isomer, so that only 50% of *S*-allyl-DL-cysteine sulphoxide was converted into allicin even after incubation for 80 min. (Stoll & Seebeck, 1949*b*).

These results have been confirmed with DL-alliin. When the alliinase preparation was mixed with an equal volume of 0·05 M alliin solution, 50% of the alliin was converted into allicin within 5 min. and no more allicin or ammonia was formed during periods up to 90 min. Allicin is unstable and was therefore prepared just before use by incubating at 37° for 15 min. the calculated quantity of alliin solution with an equal volume of alliinase. The preparation contained, in addition to allicin, unchanged D-alliin, alliinase, ammonia and pyruvic acid, so that for study of the effect of allicin on the enzymes, control experiments with equivalent or greater concentrations of alliin, alliinase, ammonia and pyruvic acid were necessary.

Effect of alliin, allicin and alliinase on enzymes

Alliin (0·0057 M) and alliinase (0·3 ml. of the standard preparation) when tested separately had no effect on the succinic oxidase system. When, however, they were incubated together the allicin produced powerfully inhibited the succinic oxidase system; the inhibition produced by 0·00029 M allicin was approximately equivalent to that produced by a solution obtained by extracting garlic with 50 times its weight of water (Fig. 1).

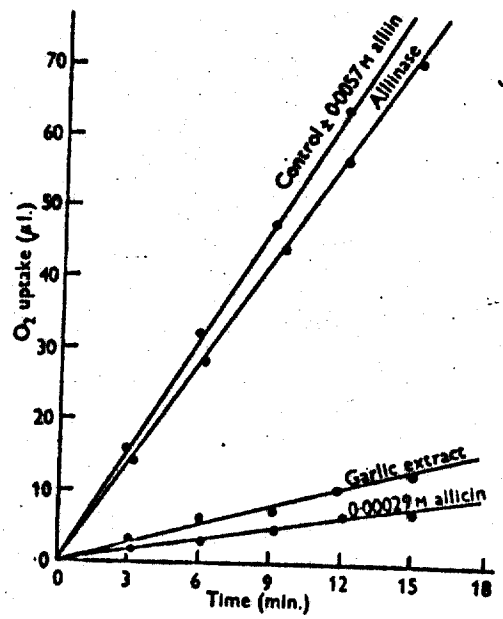

Fig. 1. Comparison of the effects of alliin, allicin, alliinase and crude garlic extracts on succinic oxidase. Alliinase: the preparation described in the text diluted 10 times. Garlic extract: a solution obtained by extracting garlic with 50 times its weight of water. Substrate: 0·11 M sodium succinate.

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The inhibition of the succinic oxidase system was most likely due to inhibition of succinic dehydrogenase since cytochrome oxidase was unaffected, and succinic dehydrogenase activity, measured by the colorimetric method of Kun & Abood (1949), was strongly inhibited.

Neither allin (0·002 M) nor alliinase affected to any measurable extent any other enzyme tested. The enzymes tested in presence of allicin, however, were divisible into the two groups of Table 1 depending on whether they were inhibited or not by 0·0005 M allicin. Table 1 shows that allicin inhibits sulphydryl enzymes, and that very few members of this class of enzyme tested were unaffected. Some enzymes listed as inhibited in Table 1 were only mildly inhibited by allicin; thus 0·001 M allicin caused only 45% inhibition of tyrosinase action. The three enzymes in Table 2 were, however, extremely sensitive to allicin in very dilute solutions. The concentration of allicin used in these experiments is comparable to that ($4·5 \times 10^{-5}$ M) found by Cavallito & Bailey (1944) to inhibit completely the growth of many bacteria.

Table 1. *Summary of the effect of* 0·0005 M *allicin on some enzymes*

Enzymes inhibited	Enzymes unaffected
*Succinic dehydrogenase	Cytochrome oxidase
*Urease	Lipase
*Papain	Rennin
†Xanthine oxidase (cream)	Pepsin
†Xanthine oxidase (liver)	Trypsin
*Choline oxidase	Invertase
*Hexokinase	α-Amylase
*Cholinesterase	Esterase (serum)
*Glyoxylase	D-Amino acid oxidase
*Triose phosphate dehydrogenase	Ascorbic acid oxidase
*Alcohol dehydrogenase	Catalase
Lactic dehydrogenase	Carbonic anhydrase
Tyrosinase	*Carboxylase
Alkaline phosphatase	*Adenosine triphosphatase
	*β-Amylase

* SH enzymes. † Possibly SH enzymes.

Table 2. *Enzymes strongly inhibited by allicin*

	Substrate concn. (M)	Allicin concn. (10^{-5} M)	Inhibition (%)
Succinic dehydrogenase	0·11	33·3	90
	0·11	14·3	85
	0·11	5·6	50
Triose phosphate dehydrogenase	0·01	10·0	100
	0·01	5·0	87
	0·01	2·0	40
Xanthine oxidase (in cream) (pH 6·4)	0·005	20·0	95
	0·005	10·0	71
	0·005	5·0	36

Effect of allicin on xanthine oxidase

The xanthine oxidase activity of cream was powerfully inhibited by allicin (Table 2). Xanthine oxidase may be an SH-enzyme (Mackworth, 1948; Harris & Hellerman, 1953) and thus fit into the general pattern. Inhibition of cream xanthine oxidase by allicin was, however, dependent on pH as shown in Fig. 2, which shows that 0·0001 M allicin completely inhibited the enzyme at pH 5·5 but had practically no effect at pH 8·2. This pH effect was the reverse of that observed with urease,

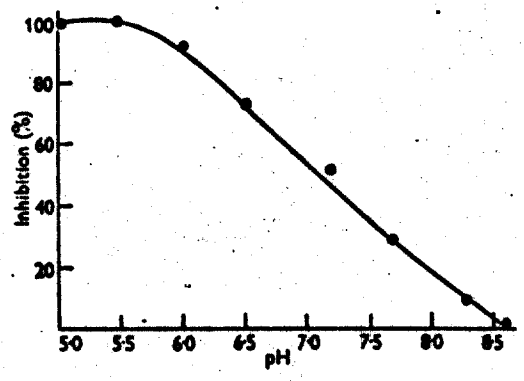

Fig. 2. Effect of pH on the inhibition of xanthine oxidase (cream) by 0·0001 M allicin. Substrate: 0·005 M hypoxanthine.

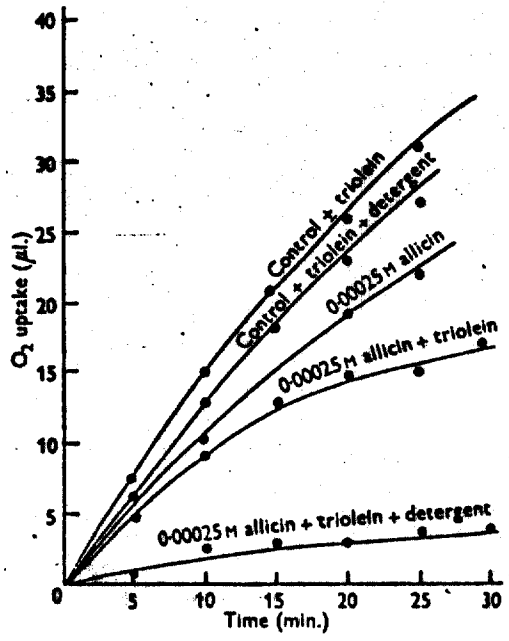

Fig. 3. Effect of allicin, triolein and a detergent on purified xanthine oxidase (Fraction M5). Triolein: 0·1 ml./3·0 ml. of total aqueous phase. Detergent: 3% (w/v) Lubrol M.O. Substrate: 0·005 M hypoxanthine.

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when inhibition by allicin increased with increasing alkalinity (Fig. 5).

Although allicin inhibited oxygen uptake by xanthine oxidase it did not affect the dehydrogenase activity as measured by the methylene-blue technique. In addition, methylene blue protected xanthine oxidase against inhibition by allicin of oxygen uptake. Thus 0·0167 M methylene blue completely abolished inhibition by 0·0001 M allicin, and the protective action was still detectable with 0·0001 M methylene blue.

When cream extracted with phosphate buffer was used as a source of xanthine oxidase, inhibition was produced by allicin but the inhibitory effect was less than that observed with cream. The buttermilk (M1), filtrate enzyme (M2) and crystalline enzymes (M7) of Avis *et al.* (1955) were practically unaffected by 0·0001 M allicin at any pH tested. This was due to the complete absence of fat in these preparations. The addition of a small quantity of triolein produced a marked increase in the inhibitory effect, and this was enhanced if the enzyme and triolein were first emulsified with a detergent before addition of the allicin (Fig. 3). The xanthine-oxidase activity of rat liver was also completely inhibited by 0·00025 M allicin.

Effect of allicin on yeast and tissue respiration

Yeast fermentation of glucose, the respiration of yeast in the presence of glucose and the endogenous respiration of rat-liver homogenate and rat-muscle homogenate were all inhibited by allicin but unaffected by alliin. Thus 0·00025 M allicin caused 43% inhibition of yeast respiration and 50% inhibition of the respiration of rat-muscle or liver homogenate.

Effect of garlic extracts on enzymes

Aqueous extracts of crushed garlic bulb were tested on urease, succinic oxidase, amylase, trypsin and lipase. Garlic was extracted with 5 times its own weight of water, and the juice was filtered. Succinic oxidase was completely inhibited by this extract diluted a further 10 times and urease was 67% inhibited. Trypsin, amylase and lipase were unaffected. The pattern of the effect of crude garlic extract on these enzymes was thus parallel to the effect of allicin. These results are in agreement with those of Szymona (1952) and Vinokurow *et al.* (1947).

Protection of enzymes against inhibition by allicin

Various compounds were tested for their ability to protect succinic dehydrogenase and urease against inhibition by allicin. The sulphydryl compounds cysteine, glutathione and BAL (2:3-dimercaptopropanol) were good protective agents, and serum had a weak protective action. Alanine, glycine, glutamic acid, arginine, histidine, phenylalanine, gelatin, native or denatured ovalbumin, and serum albumin had no effect. Cysteine (0·01 M) completely protected succinic oxidase against 0·00025 M allicin if mixed with the allicin before addition of enzyme, and 0·001 M, but not 0·0001 M, cysteine completely protected against 0·00014 M allicin. Glutathione protected to an approximately equal extent when used in the same concentrations as the cysteine. If, however, the allicin had had prior contact with the enzyme for even a short time, cysteine or glutathione restored only a small fraction of the enzymic activity.

Combination of allicin with these sulphydryl enzymes was extremely rapid. Thus 0·00025 M allicin produced 81% inhibition of succinic oxidase if added with the substrate, and 92% inhibition if the enzyme was in contact with allicin for 14 min. before addition of substrate. Prolonged periods of contact between allicin and enzyme did not significantly increase the inhibition. Similar results were obtained for urease by Agarwala *et al.* (1952). Addition of glutathione or cysteine to succinic oxidase at different times after addition of allicin showed that some regeneration of enzyme activity was obtainable after short contact periods, but little activity was recoverable after enzyme and allicin had been in contact for 15 min. (Fig. 4).

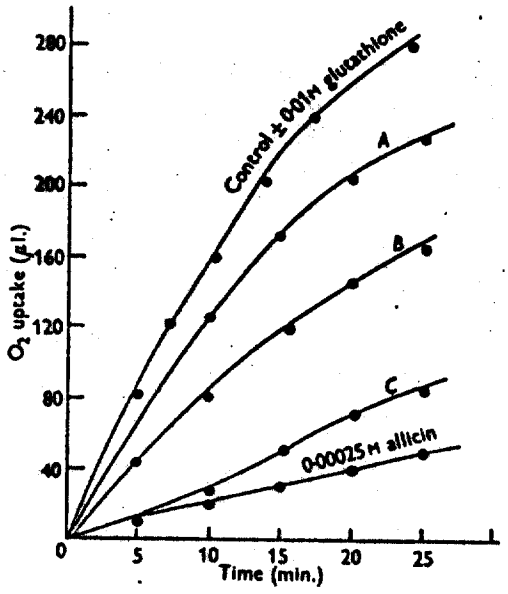

Fig. 4. Effect of glutathione (0·01 M) on the inhibition of succinic oxidase by allicin (0·00025 M). *A*, glutathione mixed with enzyme before addition of allicin; *B*, glutathione added after enzyme and allicin had been in contact for 5 min.; *C*, glutathione added after enzyme and allicin had been in contact for 15 min. Substrate: 0·11 M sodium succinate.

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The effect of pH on the inhibition of urease by allicin and on the protective effect of 0·002 M cysteine was investigated. The extent of inhibition by 0·00025 M allicin decreased slightly as the pH decreased from pH 7·0 to 5·0, but the protection by 0·002 M cysteine, which was complete at pH values greater than 6·5, was much reduced at pH 5·0 (Fig. 5).

Effect of compounds related to allicin on enzymes

Urease and succinic oxidase were used for testing the effect of several compounds with a structure related to that of allicin. Results obtained with succinic oxidase (Table 3) showed that allicin was a very powerful inhibitor but that diallyl disulphide, diallyl sulphide or diallyl sulphoxide had no effect under the experimental conditions.

Synthetic allicin

Synthetic allicin inhibited urease and succinic oxidase to approximately the same extent as did allicin prepared enzymically.

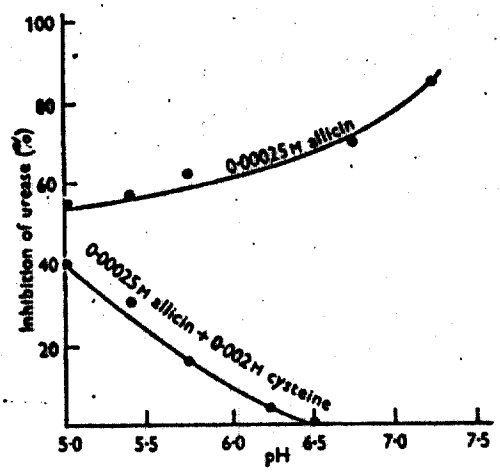

Fig. 5. Effect of pH on the inhibition of urease by 0·00025 M allicin and on the effect of the addition of 0·002 M cysteine. Cysteine and allicin were in contact for 5 min. at specified pH before addition of urease. Substrate: 0·004 M urea.

DISCUSSION

The experiments described leave little doubt that enzyme inhibition observed with crude garlic extracts is caused by allicin. Table 1 shows that with few exceptions SH-enzymes were inhibited and that most other enzymes were not affected. The cause of the inhibition of the enzymes which are not normally regarded as SH-enzymes, and the nature of the groups in the enzyme molecule attacked by allicin, are not yet clear. Rao *et al.* (1946) found that sweet potato β-amylase was 7% inhibited by 0·00031 M allicin after contact for 5 min. but that the inhibition increased to 26% after contact for 30 min. In the present work no inhibition of unpurified malt β-amylase by 0·0005 M allicin could be detected after contact for 15 min. A likely interpretation of these experiments is that the SH groups of β-amylase are much less accessible to or reactive with allicin than are those of succinic dehydrogenase, where maximum inhibition is rapidly obtained. A similar explanation is probable for the failure of allicin to inhibit carboxylase and adenosine triphosphatase, which are usually regarded as sulphydryl enzymes (Barron, 1951).

Cavallito & Bailey (1944) found that 1:125 000 ($\simeq 4{\cdot}5 \times 10^{-6}$ M) allicin completely inhibited the growth of fourteen out of fifteen strains of bacteria tested, but that half that concentration had no effect on any strain tested. In the present work powerful inhibition of sulphydryl enzymes was observed with $5{\cdot}6 \times 10^{-5}$ M allicin for the succinic oxidase system, 2×10^{-5} M for triose phosphate dehydrogenase and 5×10^{-5} M for xanthine oxidase. It is therefore clear that some vital oxidation enzymes are inhibited in a concentration that would adequately explain the bactericidal action of allicin.

Small *et al.* (1947) have, by the synthesis of a series of alkylthiosulphinates of general formula R–SO–S–R, demonstrated that the –SO–S– group was essential for bactericidal action. The results of Table 3 show that the –SO–S– grouping is also essential for inhibition of the succinic-oxidase system and that the –S–S–, –S– and –SO– groupings were not effective. Similar results were obtained

Table 3. *Comparison of the effect of allicin and related compounds on the succinic oxidase system*

Enzyme and compound in contact for 30 min. before start of experiment. Sodium succinate (0·11 M) was used as the substrate in each experiment.

	Formula ($R = -CH_2.CH{:}CH_2$)	Concn. (mM)	Inhibition (%)
*Diallyl sulphide	R.S.R	3·3	0
*Diallyl disulphide	R.S.S.R	3·3	0
Diallyl sulphoxide	R.SO.R	3·3	0
Alliin	$R.SO.CH_2.CH(NH_2).CO_2H$	4·0	0
Allicin	R.S.SO.R	0·14	84

* Used as a suspension.

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with urease. There is thus a close relationship between the grouping essential for bactericidal action and that for enzyme inhibition. These authors also observed that increasing the length of chain in the R group increased the activity of the thiosulphinate against Gram-positive organisms, and thus, for example, the diallyl compound, allicin, was more effective than the dimethyl derivative. They suggested that the increased length of the R chain rendered the compound more lipid-soluble, and the thiosulphinate, partially dissolved in the lipid portion of a lipoprotein, would thus more effectively attack the protein SH groups. This hypothesis receives strong support from the results of the present experiments with xanthine oxidase. This enzyme was practically unaffected by allicin when purified and free from fat, but it was powerfully inhibited in the natural state when absorbed by the liposomes of the milk. The purified enzyme was inhibited when added to fat artificially emulsified. This is a rare example of an enzyme which is more powerfully inhibited in the crude than in the purified state. In the case of xanthine oxidase in cream the fat is likely to act by dissolving the allyl groups and bringing the allicin into close proximity with the enzyme.

Cavallito (1946) has classified bactericidal agents attacking SH compounds into three groups, depending on their specificity. Members of the first group, which attack nearly all sulphydryl compounds, react rapidly with the SH group of cysteine and cysteine peptides. Allicin is a member of this group. The second, and third group which includes penicillin, are more specific and only attack some SH groups. Cavallito *et al.* (1944) concluded that the main product of the action of cysteine on allicin was *S*-thioallylcysteine:

$$C_3H_5.S.S.CH_2.CH(NH_2).CO_2H.$$

Rao *et al.* (1946) state that *S*-thioallylcysteine is quantitatively formed. Similar combination of allicin might occur with enzyme SH groups, and the difficulty of reversing the inhibition is in accord with this possibility. On the other hand, the labile oxygen atom of allicin may simply oxidize the SH groups to –S–S–. The effect of pH on the inhibition of urease by allicin and the protective action of cysteine shows that inhibition of urease, although decreasing with increasing acidity, does so much less than does the protection by cysteine (Fig. 5). A probable interpretation of these results is that the SH group of cysteine is much less readily attacked than are the SH groups of urease as the pH is progressively lowered. The effect of pH on the inhibition of xanthine oxidase by allicin probably bears no relation to the case of urease, and is likely to be connected with the effect of pH on the state of the lipoprotein complex of the cream.

SUMMARY

1. All enzyme inhibitory effects observed with garlic extracts may be explained by the presence of allicin in them.

2. Alliin (0·002 M) did not inhibit any enzyme tested, but 0·0005 M allicin inhibited nearly all sulphydryl enzymes but very few non-sulphydryl enzymes tested. Some enzymes, such as triosephosphate dehydrogenase, were inhibited by 0·00005 M allicin.

3. Sulphydryl enzymes could be protected by cysteine or glutathione against inhibition by allicin, but only partial recovery of enzyme activity was obtainable after the allicin and enzyme had been in contact for a short period.

4. Inhibition of sulphydryl enzymes has been shown to be associated with the presence of the –SO–S– grouping and not the –SO–, –S–S– or –S– groups.

The author wishes to express his thanks to Professor A. Wormall for advice and interest in this work and to Miss G. Fisher for technical assistance. Gifts of alliin from Sandoz Ltd. and of several samples of pure xanthine oxidase from Professor F. Bergel are gratefully acknowledged.

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Pathways of Glucose Catabolism in Rat Liver in Alloxan Diabetes and Hyperthyroidism

By GERTRUDE E. GLOCK, PATRICIA McLEAN AND J. K. WHITEHEAD
Courtauld Institute of Biochemistry and Barnato–Joel Laboratories, Middlesex Hospital, London, W. 1

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A previous publication (Glock & McLean, 1955) reported striking alterations in levels of activity of enzymes of the hexose monophosphate oxidative pathway of glucose metabolism both in alloxan diabetes and hyperthyroidism. It was found that levels of activity of both glucose 6-phosphate and 6-phosphogluconate dehydrogenases were markedly reduced in the livers of diabetic rats and greatly increased after thyroxine treatment. In an attempt to substantiate these findings and to assess the relative importance of glycolytic and non-glycolytic pathways of carbohydrate metabolism, the utilization of [1-^{14}C]glucose and [6-^{14}C]glucose by liver slices from normal, diabetic and hyperthyroid rats has been determined by measuring the conversion of these labelled substrates into $^{14}CO_2$. In addition to these isotope measurements, the levels of activity of glucose 6-phosphatase and phosphoglucose isomerase have also been determined, since the former is one of the factors regulating the availability of glucose 6-phosphate, the common substrate for both metabolic pathways, whilst striking alterations in activity of phosphoglucose isomerase might be expected to influence the proportion of glucose 6-phosphate metabolized via the glycolytic route.

EXPERIMENTAL

Materials

D-*Glucose 6-phosphate* (*G 6-P*). This was a preparation of the heptahydrate of the barium salt obtained from Sigma Chemical Co.

6-*Phosphogluconate* (*6-PG*). This was a preparation of the barium salt used previously (Glock & McLean, 1953).

[1-^{14}C]*Glucose and* [6-^{14}C]*glucose*. [1-^{14}C]Glucose was obtained from the Radiochemical Centre, Amersham, and [6-^{14}C]glucose from Dr H. S. Isbell, National Bureau of Standards, Washington.

Methods

Estimation of G 6-P dehydrogenase and 6-PG dehydrogenase activities. These were determined spectrophotometrically by following the rate of reduction of triphosphopyridine nucleotide (TPN) at 340 mμ. in 1 cm. cells in a Hilger Uvispek spectrophotometer as described previously (Glock & McLean, 1953). In the assay of G 6-P dehydrogenase activity, the reaction mixture consisted of 0·5 ml. of 0·25 M glycylglycine (pH 7·6), 0·5 ml. of 0·1 M-$MgCl_2$, 0·1 ml. of 6-PG dehydrogenase prepared from rat liver by fractionation with $(NH_4)_2SO_4$, followed by dialysis (Glock & McLean, 1953), 0·1 ml. of liver supernatant and 0·2 mg. of TPN in a total volume of 2·4 ml. The reaction was started by the addition of 0·1 ml. of 0·05 M G 6-P to both cells, the blank being devoid of TPN. In the assay of 6-PG dehydrogenase activity, the $(NH_4)_2SO_4$ liver fraction was omitted, G 6-P was replaced by 0·1 ml. of 0·05 M 6-PG and the reaction was carried out in glycylglycine buffer at both pH 7·6 and pH 9·0. A unit of enzyme activity is defined as the quantity of enzyme which reduces 0·01 μmole of TPN/min. at 20°.

Estimation of glucose 6-phosphatase activity. This was determined according to Dave, Pressman, Gianetto, Wattiaux & Applemans (1955). The incubation mixture contained 0·04 M G 6-P, 0·007 M histidine, 0·001 M ethylenediaminetetraacetate (pH 6·5) and 0·2 ml. of 10% (w/v) liver homogenate in a total volume of 1 ml. After incubation for 10 min. at 37°, the reaction was stopped by the addition of an equal volume of 10% trichloroacetic acid, and inorganic P was determined in the filtrate by the method of Fiske & Subbarow (1925). A unit of enzyme activity is defined as the quantity of enzyme which hydrolyses 1 μmole of G 6-P/min. at 37°.

Estimation of phosphoglucose isomerase activity. This assay was based on that described by Slein (1955) for muscle phosphoglucose isomerase, fructose 6-phosphate formation being determined colorimetrically by the method of Roe (1934), which gives approximately 65% of the colour of free fructose. The incubation mixture contained 4 μmoles of G 6-P, 20 μmoles of glycylglycine (pH 7·6), and 0·1 ml. of 0·1% liver homogenate in a total volume of 0·5 ml. The homogenate was prepared in ice-cold isotonic KCl (0·15 M)

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INHIBITION OF PHOSPHOGLUCOMUTASE BY AN EXTRACT FROM GARLIC[1]

R. D. HART,[2] R. HALDIMAN, G. A. GRIES,[2] AND B. J. ROGERS

Introduction

Eyster (5) in 1949 demonstrated the presence of an inhibitor of starch synthesis in the expressed juice from plants in several monocotyledonous families. The juice of onion, wild garlic, day lily, and jonquil, when diluted 1:1 with 1 *M* glucose solution, completely inhibited the formation of starch in squares of etiolated corn leaves floated on the solution.

Eyster's observation could be explained on the basis of either inhibition of glucose uptake or inhibition of one of the enzymes involved in starch synthesis and degradation. By techniques similar to Eyster's we have demonstrated that the juice of both onion (*Allium cepa*) and wild garlic (*A. vineale*) inhibits not only synthesis but also the hydrolysis of starch in floating leaf discs. These findings indicate that the extract did not affect glucose uptake but must have acted directly on one of the enzymes. We have further demonstrated that this inhibitor acts specifically on the enzyme phosphoglucomutase.

[1] Contribution from the Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana. Journal Paper no. 1486, Purdue University Agricultural Experiment Station.

[2] Present addresses: AmChem Products, Inc., Ambler, Pennsylvania, and Department of Plant Pathology, University of Arizona, Tucson, Arizona, respectively.

Material and methods

The inhibitor was concentrated from wild garlic bulbs by a series of extractions and steam distillations. An aqueous brei was filtered through cheesecloth or centrifuged at low speed to eliminate the large fragments of plant material. The active fraction

was extracted from the filtrate with chloroform and concentrated again in water by *in vacuo* distillation of the chloroform at reduced temperature. The active fraction could be steam-distilled from the solution when acidified with H_3PO_4. Volatile basic and neutral compounds were removed by basic distillation. After being redistilled from acid solution, the inhibitor was extracted into ethyl ether. A small amount of highly active oily material remained following the evaporation of the ether. A water solution of this material was used in the enzyme studies.

Starch phosphorylase was prepared from potatoes by the method of GREEN and STUMPF (6). The reaction mixture contained 0.3 ml. of crude potato phosphorylase preparation, 0.4 ml. of 0.06 *M* glucose-1-phosphate (G-1-P), 0.3 ml. citrate buffer (pH 6.1), and either deionized water or inhibitor solution to make a final volume of 2.0 ml. The reaction was carried out at room temperature; starch formation was assayed by the iodine test.

A commercial preparation (Sigma Chemical Corp.) of yeast hexokinase was used to study the step from glucose to glucose-6-phosphate (G-6-P). The enzyme activity was equivalent to 75,000 K.M. units gm at 25° C. The reaction mixture contained 30 mg. glucose, 2 mg. hexokinase, 1.0 ml. veronal buffer (pH 7.4), 1.0 ml. of 0.01 *M* adenosine triphosphate (sodium salt), 0.1 ml. of 1 *M* $MgCl_2$, and 1.0 ml. of either deionized water or the active inhibitor solution. The reaction was carried out at 23° C. Aliquots taken at 2, 4, and 20 hours were chromatographed by the technique of BANDURSKI and AXELROD (1), and sugar phosphates were detected by the method of HANES and ISHERWOOD (7).

Phosphoglucomutase, the enzyme which catalyzes the interconversion of G-1-P and G-6-P, was prepared from potatoes by the method of BOSER (2). Since the equilibrium mixture contains 95% G-6-P, the conversion to this sugar was studied. The reaction mixture used contained 0.3 ml. of the crude enzyme preparation, 0.3 ml. of 0.06 *M* G-1-P, 0.2 ml. of citrate buffer (pH 6.4), 0.1 ml. of 1 *M* $MgCl_2$, and 0.3 ml. of either deionized water or the active inhibitor solution. The reaction was carried out at 33° C. Aliquots were tested for the sugar phosphates at regular intervals by the techniques cited above.

Results

Preliminary studies indicated the inhibitor to be a small molecule, since it could be dialyzed. It is soluble in water, benzene, chloroform, and ethyl ether and can be concentrated by extraction in such solvents followed by *in vacuo* evaporation at low temperatures. The activity of the inhibitor disappears upon exposure of the *Allium* bulbs at 70° C. for 30 hours. The extract can be autoclaved in a sealed tube at 15 lb/sq in for 30 minutes, however, without loss of activity. The inhibitor apparently may be held indefinitely at −20° C. at any stage of purification.

The reaction mixture which contained starch phosphorylase was found to produce starch within 20 minutes. The substitution of active solutions of the inhibitor for water did not reduce starch production. This step obviously is not affected by the inhibitor.

In the study of hexokinase, it was found that G-6-P was formed readily in the reaction mixture used and could be detected chromatographically in the aliquot taken at 2 hours. There was no apparent diminution in formation, when the inhibitor solution replaced water in the reaction mixture.

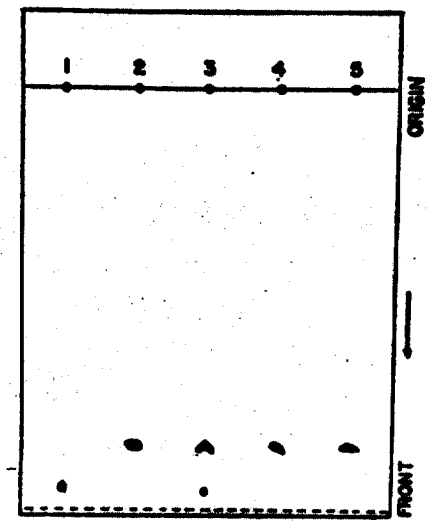

FIG. 1.—Representation of a chromatogram developed with methanol, 88% formic acid, and water (80:15:5) and detected by the method of HANES and ISHERWOOD (7). Initial spots at origin were as follows: 1, G-6-P freshly dissolved; 2, G-1-P freshly dissolved; 3, enzyme preparation, G-1-P, citrate buffer (pH 6.4), MgCl, and deionized water incubated at 33° C. for 2 hours; 4, same mixture as 3, freshly prepared; 5, same mixture as 3 but with garlic extract substituted for deionized water and incubated at 33° C. for 2 hours.

After completion of the above two enzyme studies, it was apparent that the enzyme involved in the inhibition of starch synthesis was phosphoglucomutase, and this was found to be the case. The results of one experiment are shown in figure 1. A spot on the chromatogram corresponding to G-6-P appeared after 2 hours from the mixture containing G-1-P and the enzyme. When the deionized water of the mixture was replaced by the active inhibitor solution from wild garlic, no G-6-P could be detected.

Discussion

The carbohydrates of garlic are glucose, fructose, sucrose, and fructosans (10); no starch has been found, although apparently starch phosphorylase is

present (9). The occurrence of sucrose and fructosans presents a dilemma in view of the facts that known systems for the synthesis of sucrose require G-1-P and that sucrose is required for fructosan synthesis (8). Either an obscure pathway exists in these plants for the synthesis of sucrose from fructose phosphates and glucose, or the inhibitor is isolated in the vacuoles of the cells. If the latter alternative is correct, it should be possible for the garlic plant to produce starch as well as sucrose. In any event, glycolysis and subsequent reactions could go on normally in these plants, since fructose-6-phosphate is produced in photosynthesis and could be utilized directly.

It is interesting to speculate that an inhibitor of this sort is important in the differentiation of plants into starch-producers and fructosan-producers, but this speculation is untenable unless a pathway which does not require G-1-P exists for the synthesis of fructosans.

The relation between this inhibitor of starch synthesis and the antibacterial substance (allicin) extracted from onions by CAVALLITO *et al.* (3, 4) is not clear. Although the solubilities of the two compounds are similar, we have not been able to maintain high activity for both properties in purification procedure.

Summary

We have demonstrated that the inhibitor of starch synthesis found in onion and garlic acts on the enzyme phosphoglucomutase.

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A SIMPLE APPARATUS FOR MEASUREMENT OF SEEDLING GROWTH UNDER CONTROLLED TEMPERATURE

P. J. DUBASH AND B. A. KHAN

At least two methods exist for measurement of root elongation of intact seedlings by means of the microscope (1, 2). Temperature regulation is not incorporated in the units described, however, but is maintained by general air conditioning or some such means. The following simple assembly, where the required temperature is maintained by means of hot or cold water, can be used in laboratories without air conditioning.

The apparatus (fig. 1), constructed from Pyrex glass, consists of an outer cylindrical jacket (*A*), 5 × 25 cm., the upper end of which is open and the lower end fitted with a stopper with three holes. This jacket contains a thistle funnel (2.5 × 5 cm.), the stem (1 × 28 cm.) of which emerges through the central hole in the cork. The distal end of the stem is drawn out into a jet fitted with a stopcock (*B*). An inlet tube 1 cm. in diameter passes through another of the holes in the cork and is connected by a rubber tube to a 500-ml. water reservoir (*C*). Another glass tube, 1 × 25 cm., inserted in the remaining hole as an outlet, is fitted with a rubber tube and pinchcock. The desired temperature can be obtained by filling the outer jacket (*A*) with water at a suitable temperature from the reservoir (*C*). It is maintained by draining out some of the water and adding hot or cold water as needed. The thermometer is not shown in the figure. It has been possible to maintain the temperature within ±0.5° C.

The apparatus is prepared for use as follows: Seeds are surface-sterilized with 0.2% mercuric chloride, washed, and then germinated on moist filter paper in large Petri dishes. After germination, the seedling is moved to the apparatus where it is supported in the wider end of the thistle funnel by a swab of moist cotton with its root extending into the stem. The growth medium, which fills only the stem of the funnel, can be renewed at regular intervals

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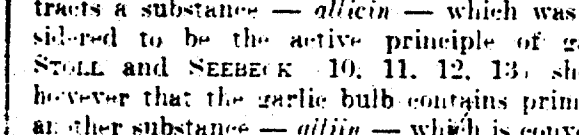
GARLIC AS AN INHIBITOR FOR SPONTANEOUS TUMORS IN MICE

F. KRÖNING *

INTRODUCTION

For more than 2000 years, *garlic*, the bulb of *Allium sativum L.* is used as a medicinal drug. It is believed to inhibit arterial sclerosis in man, although experiments with animals could not confirm this meaning. Another use of garlic is based on its bactericidal effect. (LEHMANN (7), DI PAOLO and CARRUTHERS (3)).

CAVALITTO et al. (2) isolated from garlic extracts a substance — *allicin* — which was considered to be the active principle of garlic. STOLL and SEEBECK (10, 11, 12, 13) showed however that the garlic bulb contains primarily another substance — *alliin* — which is converted into allicin by an enzyme — *allinase* —, which is set free after the bulbs are minced:



v. EULER and LINDEMANN (4) described alliin as a tumor inhibitor, but NOWIKOWA, LEVY and CHOCHLOW (8) and DI PAOLO and CARRUTHERS (3) could not verify this concept. In our experiments alliin was also ineffective.

WEISBERGER and PENSKY (14), ROCHETTA (9) and DI PAOLO and CARRUTHERS (3) found allicin to be an inhibitor for tumor growth.

The results of WEISBERGER and PENSKY (14) and DI PAOLO and CARRUTHERS (3) encouraged us to attempt the contents of garlic bulbs as a *prophylacticum* against tumor development in mouse strains with hereditary tumors.

The experiments were granted by the firm Woelm-Eschwege and were undertaken in collaboration of its biochemical Laboratory (Prof. Dr. R. Mentzel).

MATERIALS AND METHODS

We employed AKR J-mice and C3H HeJ-mice from the Zentralinstitut für Versuchstierzucht in Hannover-Linden. We received the mice when they were 30-35 days old.

We had three series with different diets:

1. diet without addition (control);
2. diet with fresh garlic (with allicin);
3. diet without allinase in the garlic (containing only alliin).

The diet was prepared every week. 20 males and 20 females were in each series. The diets and water were offered ad libitum. The daily consumption of the diet was controlled.

The experiments are not closed. Here are presented the results 500 days after the beginning.

RESULTS

The garlic diet was at once accepted despite the smell of this substance. After 150 days in the AKR-strain the first leukemias appeared, the mice being at this time 180 days old. There seems to be no difference in the three series neither in the time of appearing of the tumors, nor in their frequency. After 360 days the remaining mice were killed, all showed leukemic tumors.

Males and females showed the same percentage of leukemias, the garlic preparations were ineffective on the developing leukemia in the AKR-strain.

The C3H mice differ from this observation very markedly. Until now no mammary-tumor was recognized in the females, which received the diet with the fresh garlic, but 50% of the females died with tumors, which eat the standard diet and 60% which eat the garlic without smell.

There exists a complete inhibition of the mammary tumors in the females C3H-mice by fresh garlic. A good deal of the mice, which developed a mammary tumor, had also a leukemic tumor: thymus tumors and (or) mesenterical tumors.

Besides this two forms of tumors we found one lung tumor in a male which received the diet with the desodorized garlic. One male in the series with fresh garlic had a carcinoma of the dorsal skin. This shows possibly, that also this tumor type is not influenced by garlic. Till now hepatomas were not observed.

The number of mice which died from other causes is the same in the series which got the standard diet and the diet with desodorized

* From the Medizinische Forschungsanstalt of the Max-Planck Gesellschaft, Bismarckstrasse 10, 34 Göttingen, Germany.

MEDICAL RESEARCH

garlic. Some more animals died which received the fresh garlic. But this difference seems to be neglectible.

The addition of the fresh garlic to our mouse diet completely inhibited the formation of mammary tumors in the C3H-mice. It was quite ineffective against the leukemias of virus origin of the AKR-strain.

Garlic cannot act on the Bittner's mammary tumor agent, because C3H-mice which are free of the agent develop mammary tumors although in a lesser percentage.

The point of attack of garlic therefore cannot influence the mammary tumor agent, there must be another still unknown pathway. It is needless to speculate about this point of attack at this time.

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SUMMARY

The feeding of fresh garlic inhibits completely the development of mammary tumors in female C3H-mice. Garlic, in which the enzyme allinase was destroyed, was ineffective. Therefore it is likely that allicin is the active principle of garlic and not its precursor alliin.

Against the leukemias of the AKR-strain garlic feeding was without success.

RÉSUMÉ

L'addition d'ail frais à la nourriture inhibe complètement l'apparition de tumeurs de la mamelle chez les souris femelles C3H. L'ail, dans lequel l'allinase est détruite n'a aucune action. Dès lors, il semblerait que l'allicine est le principe actif de l'ail et non son précurseur l'alliine.

La nourriture à l'ail s'est avérée sans succès contre la leucémie de la souche AKR.

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#141

[GANN, 55, 325~329; August, 1964]

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CYTOLOGICAL EFFECT OF CHEMICALS ON TUMORS. XXIII. INFLUENCE OF CRUDE EXTRACTS FROM GARLIC AND SOME RELATED SPECIES ON MTK-SARCOMA III*[1]

(Plate XLVIII)

Yanagi KIMURA and Kôtaro YAMAMOTO
(Zoological Institute, Hokkaido University*[1])

Synopsis

This paper describes the effect of crude extracts from garlic (*Allium sativum* f. *pekinense*) and its related species, *A. bakeri*, *A. cepa*, *A. fistulosum*, and *A. victorialis* subsp. *platyphyllum*, on MTK-sarcoma III, an ascites sarcoma of albino rats. Of the plants tested, the extract from garlic exerted the most marked antimitotic effect on tumor cells. Cytologically the effects of this plant were much like those induced by colchicine, by producing blockage of metaphase cells and scattering, as well as abnormal condensation of metaphase chromosomes.

Extracts from *A. victorialis* and *A. cepa* produced effects similar to those of garlic, though their action was not very severe. The cell-damaging action of the extracts from *A. bakeri* and *A. fistulosum* was much less than extracts from above plants.

Extract from garlic (*Allium sativum* f. *pekinense*) has been known to contain a powerful bactericidal agent, allyl allylthiosulfinite (allicin).[3,6] Weisberger and Pensky[12,13] observed a marked tumor-inhibiting effect of garlic enzyme on malignant cells in direct contact with this compound prior to inoculation. Of particular interest is that garlic and saffron (*Colchicum autumnale*) from which colchicine is isolated belong to the same family, Liliaceae.

The present work was undertaken to observe any possible antimitotic effect of crude extracts from garlic as well as from some related species on transplantable rat tumor cells.

MATERIALS AND METHODS

Plants chosen here for experiments are solely members of the Allioideae, i.e., garlic (*Allium sativum* f. *pekinense*), scallion (*A. bakeri*), onion (*A. cepa*), Japanese

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*[1] Kita-8-jo, Sapporo, Hokkaido (木村やなぎ, 山本興太郎).

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leek (*A. fistulosum*), and *A. victorialis* subsp. *platyphyllum* (a kind of wild garlic) all of which are common vegetables in Japan. *A. victorialis* subsp. *platyphyllum* was collected on Zenibako Pass (Prov. Shiribeshi, Hokkaido) during June to September, 1963, and the others were obtained from local stores. Bulbs of the plants were used in most cases. In the species which do not form bulbs, the tuber or corm provided the material for the study. The methods of preparations for plant extracts were essentially the same as described previously.[5] The MTK-sarcoma III, an ascites tumor of albino rats, successively transferred to Wistar rats weighing 110~140 g, was used for this study. Observations were carried out on tumor cells at appropriate intervals following a single intraperitoneal injection of the extracts at doses of 1, 3, 5, and 10 mg/100 g, starting on the 4th day of tumor transfer. Preparations were made according to the acetic dahlia squash method.[7]

Results

Of the species tested, extracts from garlic, onion, and *A. victorialis* subsp. *platyphyllum* induced blockage of metaphase and contraction or scattering of metaphase chromosomes, in a like manner commonly noted in cells treated with colchicine in a decreasing order. Effect of extracts from the other two species, scallion and Japanese leek on tumor cells was much less than those from the above species. Garlic extract produced a marked damage to tumor cells within 1 hour after injection (Fig. 1). In a manner similar to that of colchicine, the garlic extract first damaged the chromosomes at metaphase. Apparently the interphase cells did not show any change for about 6 hours after the injection. Division of almost all cells was blocked at metaphase and the chromosomes were irregularly condensed and scattered in the cell without any evidence of equatorial arrangement. Damage to cells seems to proceed with time. Three to six hours following injection, the number of tumor cells with chromosomes arrested at metaphase increased considerably, with a maximal limit at 6 to 9 hours (Figs. 1 and 2). By this time irregular process of the cytoplasm or blebbing of the cell surface was also observed in the interphase cells. Degenerative changes advanced with time; the condensed chromosomes were agglutinated into irregular masses followed by pycnotic aggregation of chromatin, which led to the death of the cell. Such changes continued for about 12 hours after injection in about 50% of tumor cells observed. The accompanying photomicrographs show cell damage produced by treatment with garlic extract. Though the degree of influence was not identical in different materials, the effect of the extracts presented here appeared to follow a pattern of response similar to that of colchicine. In every experimental animal, no complete regression of the tumor has been attained. The life span of the treated animals ranged from 9 to 14 days.

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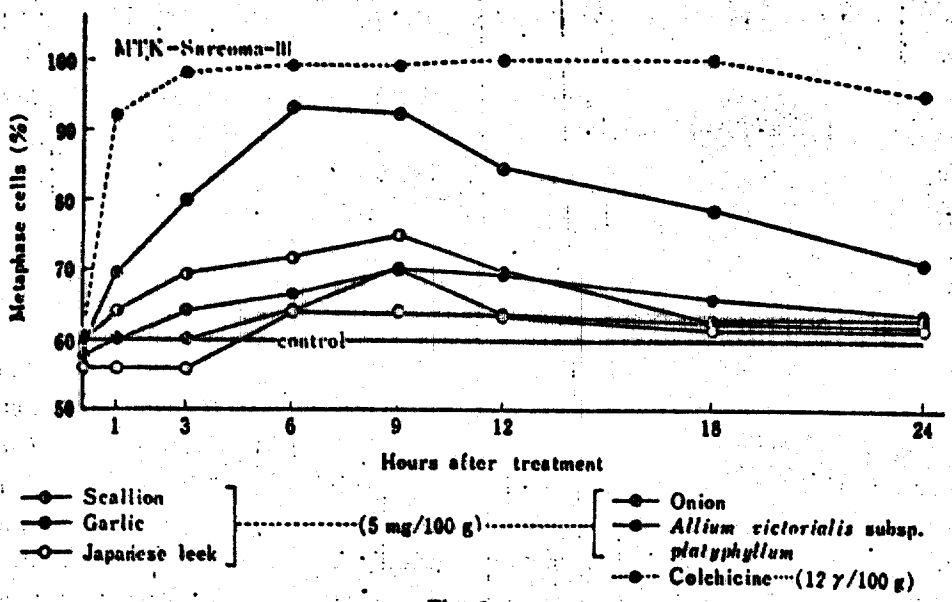

Fig. 1.

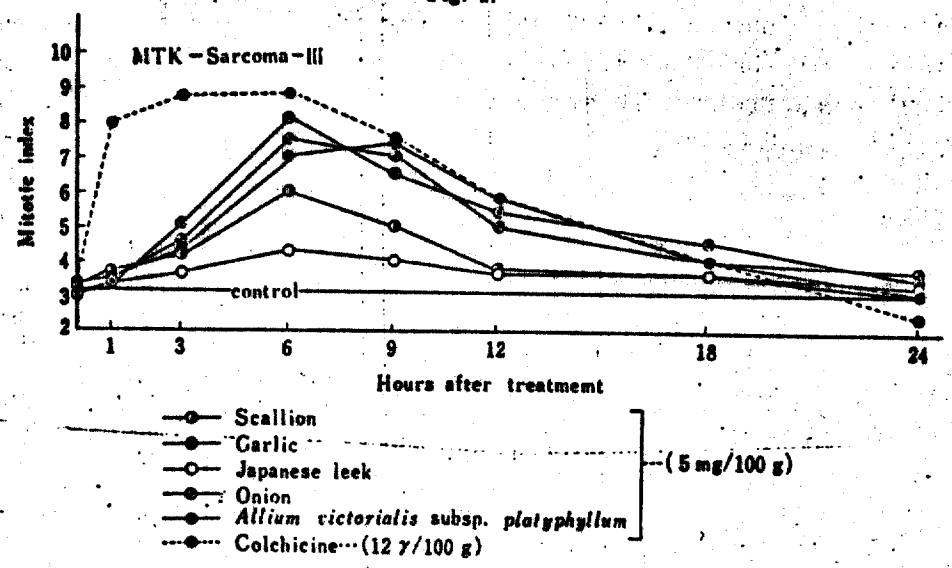

Fig. 2.

Discussion

Since the powerful bactericidal effect was found in allicin, a major component of garlic extract,[3,6] attention of many workers was attracted to the effect of allicin on bacteria.[2,4,8,9,14] Willis[4] has shown that this agent inhibits many sulfhydryl (-SH) enzymes without influencing many of the other enzymes. Weisberger and Pensky[12,13]

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were probably the first to report the effect of this agent on neoplastic cells. They observed a striking tumor-inhibiting effect of this agent on the sarcoma-180 ascites tumor of CFW Swiss mice. They proposed that the effect of an agent which inactivates -SH groups is further suggestive of the importance of -SH metabolism in neoplasia. According to Stoll and Seebeck,[9] garlic and its related species contain alliin, a precursor of allicin, in varying quantities. Although the cytological mechanism of the garlic extract on tumor cells cannot be discussed on the basis of the present experiments, it seems probable that c-mitotic abnormalities observed here may be attributable to the reactivity of allicin to SH groups in favor of the view expressed by Weisberger and Pensky.[12] An increase in SH compounds prior to cell division has been demonstrated in a wide variety of tissues, plants, and other organisms.[2,12] Reduced SH compounds stimulate cell growth and division, whereas substances which oxidize -SH to -S-S- inhibit cell division.[12] Similar inhibition of cell division has been obtained by thiol poisons such as alkylating agents and heavy metals.[12] Abnormalities of -SH metabolism may be implicated in malignant cell growth, since a high SH content has been demonstrated in some tumor cells.[4,12]

The work on damaging effect of extracts on experimental tumors has been undertaken from early times to treat tumors in man throughout the world. A large number of plants were tested, and few were reported to have some effect against a tumor. In addition to well-known colchicine and podophyllin, substances which induce c-mitotic abnormalities in tumor cells have recently been isolated from certain species of higher plants.[1,5,11] Extensive study of this kind may lead to an effective therapeutic attack on cancer problem.

The present authors wish to express their cordial thanks to Professor Sajiro Makino for his expert guidance and for going over the munuscript.

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Explanation of Plate XLVIII

Photos 1~3. Destructive changes of MTK-sarcoma III cells following the application of garlic extract.

1. Control MTK-sarcoma III cells, just before the treatment.
2. Increase in the number of metaphase due to accumulation of arrested mitosis, 9 hours after the treatment.
3. Advanced condition of cell degeneration. Majority of cells became pycnotic with aggregation of chromatin, 12 hours after the treatment.

Photos 4~6. Metaphase chromosomes of the MTK-sarcoma III in treated and control cells.

4. Control.
5. Abnormal condensation of chromosomes, 9 hours after the treatment.
6. Stickiness of chromosomes, 9 hours after the treatment.

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#61

The Effect of Allicin from Garlic on Tumor Growth*

JOSEPH A. DIPAOLO AND CHRISTOPHER CARRUTHERS

(*Roswell Park Memorial Institute, Buffalo, N.Y.*)

SUMMARY

Allicin, alliin, and a garlic extract were tested on solid and ascites mouse tumors carried in proper hosts. Preincubation of certain concentrations of enzymatically prepared allicin with tumor cells resulted in complete inhibition of tumor growth. Single or multiple treatment, as high as 30 mg. of either allicin or alliin per kg. of mouse, did not produce any consistent and significant tumor inhibition. The garlic extract also was an ineffective tumor-inhibitor. Only allicin inhibited the yeast cells and their respiratory mutants. The stability of allinase was increased to 20–34 days by storage at −25° C.

The suspected medicinal value of garlic (*Allium sativum* L.) is now believed due to the properties of its active principle, allylthiosulfinicallyl ester, allicin (6). Allicin is produced by enzymatic degradation of alliin, S-allyl-L-cysteine-S-oxide. Inhibition of sulfhydryl enzymes is associated with the presence of the −SO−S− grouping (11). The role of sulfhydryl groups in cell division and mitoses has been reviewed by Barron (1): an increase in −SH is associated with cell division as well as growth, while agents which poison or oxidize −SH compounds inhibit cell division.

Sulfhydryl-deficient diets may inhibit tumor growth in some animals, whereas the addition of cysteine or glutathione may have the opposite effect (9, 10). Therefore, interference with sulfhydryl-carrying components might block necessary steps for division and growth of cells.

Von Euler *et al.* (7) reported that pure alliin had growth-inhibiting action on various tumors, with inconsistent complete inhibition in rats of Jensen sarcoma and of a benzpyrene-induced sarcoma. No regression of Ehrlich ascites tumors in mice was noted following a single intratumoral injection of 20 mg. of alliin. In 1957, Novinov, Levy and Chochlov (4) reported that their synthetic alliin was ineffective, at concentrations higher than those used by von Euler, on transplanted and induced tumors. Recently Weisberger and Pensky (8) reviewed some of the evidence implicating abnormalities of metabolism of sulfhydryl compounds in human leukemia and presented their own results on Sarcoma 180 (S-180) tumors in Swiss mice and the Murphy-Sturm lymphosarcoma in Wistar rats with a series of analogs of allicin. They concluded that their compounds have tumor-inhibiting effects.

The present study was undertaken to evaluate the effect of alliin and allicin itself upon mouse tumors and upon a microbiological screening system for potential tumor-inhibiting agents.

MATERIALS AND METHODS

a) Preparation of allicin.—On treatment of alliin[1]—$CH_2{=}CH-CH_2-SO-CH_2-CH(NH_2)-COOH$—with allinase, allicin ($CH_2{=}CH-CH_2-SO-S-CH_2-CH{=}CH_2$), pyruvic acid and ammonia are produced.

The enzyme allinase was prepared by the procedure of Stoll and Seebeck (5), except that the garlic bulbs were homogenized (Omnimixer) in water and the final crude enzyme preparation was redissolved not only in M/15 phosphate buffer of pH 6.4 but also in 0.9 per cent saline after the pH of the enzyme preparation was raised to 6.2–6.4 with 2 N NaOH. The activity of the alliinase was determined by measuring its capacity to split alliin (DL-alliin) to allicin, ammonia, and pyruvic acid (5). The pyruvic acid liberated was determined as the 2,4-dinitrophenyl hydrazide (5). Since alliinase is quite unstable at 0–4° C. (5), attempts were made to increase its stability by storage at lower temperatures. The results of these experiments are shown in Chart 1. Alliinase stored in M/15 phosphate buffer of pH 6.4 at 0–4° C. lost its activity rapidly (curve *A*), whereas the enzyme stored in the same buffer or in saline at −25° C. was stable for 20–34 days (curves *B* and *C*).

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[1] Authors are indebted to Sandoz Pharmaceuticals, Hanover, N.J., for the DL-alliin.

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The following procedure was employed for the enzymatic preparation of allicin. The required amounts of DL-alliin (assuming 50 per cent conversion to allicin) were dissolved in 0.9 per cent NaCl to which was added alliinase solution previously adjusted to pH 6.2–6.4 with NaOH. (Alliin in 0.9 per cent NaCl and alliinase in solution in 0.9 per cent NaCl were added in the ratio of 1.0–1.5 ml., respectively, the same ratio employed for assay of enzyme activity). The enzyme solution and alliin were mixed and immediately incubated in a water bath at 37° C. for 8–10 min. The resulting solution, which contained the allicin, was injected immediately into mice bearing tumors. In some cases the alliin was dissolved in 0.9 per cent NaCl and frozen. When allicin was needed, an alliinase preparation was thawed, added to the unfrozen alliin, and the mixture incubated at 37°C. for 8–10 min. for immediate use.

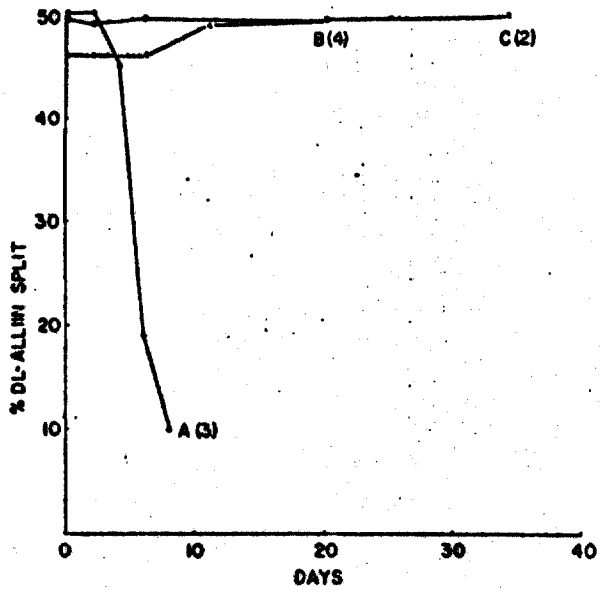

CHART 1.—Stability of alliinase. *A*. Alliinase stored in phosphate buffer, pH 6.4, at 4° C. *B*. Alliinase stored in phosphate buffer, pH 6.4, at −25° C. *C*. Alliinase stored in 0.9 per cent NaCl (adjusted to pH 6.4) at −25° C. Number of samples indicated in parentheses.

b) Preparation of water extract of garlic.—In a few experiments an aqueous extract of garlic was employed for chemotherapeutic purposes. This was made by homogenizing 10 gm. of cleaned garlic bulbs in 50 cc. H_2O (Omnimixer) for 3 min. The homogenate was then spun at 16,000 r.p.m. (International multispeed attachment and rotor No. 269) for 20 min. at 0° C. The supernatant fraction was filtered and stored at −25° C. until ready for use. As required, aliquots were thawed at room temperature.

c) Anti-tumor study.—The tumor-inhibiting effects of allicin were studied by *in vitro* preincubation with saline-suspended tumor cells for 15 min. prior to intraperitoneal inoculation into Swiss mice. Allicin, alliin, and garlic extract were tested at the maximum tolerated dose on solid and ascites mouse tumors carried in proper hosts. Concentrations greater than the maximum reported resulted in excessive deaths. The material was given by intraperitoneal or intravenous injections or oral intubation usually once daily, beginning the day after tumor transplantation and continuing for 7 or 14 days. The effect of a single injection was also studied. Control animals, usually fifteen per group, were given injections of 0.1 M phosphate buffer or saline, depending upon the solvent used in the reaction mixture. Inhibition of S-180 was determined on the 9th day following tumor transplantation by determining the average diameter of the tumors. Adenocarcinoma C-755 inhibition was determined on a wet-weight basis on the 16th day following transplantation. Inhibition of ascites tumors was determined by calculating the number of days that 90 per cent of the animals survived.

The materials were also tested for inhibition of respiratory-deficient yeast mutants and their parents, with an agar-disc assay method (3). Since a characteristic of neoplasms is an increase in the role of glycolysis, chemicals which preferentially inhibit the respiratory mutant and not the wild type yeasts may have carcinolytic or carcinostatic effects.

RESULTS

Preincubation of 5×10^6 cells of Sarcoma 180 ascites tumor or Ehrlich-2 carcinoma ascites cells with allicin (2–810 μg/0.2 ml suspension) prior to intraperitoneal injection into Swiss mice did result in complete carcinolytic action, with one exception at the 16 μg. or higher concentration per inoculum (Table 1). The corresponding controls survived less than 3 weeks.

Only slight inhibition was found in animals treated subsequent to tumor inoculation; therefore, many of the results obtained following treatment with intermediate doses of allicin are omitted from the data reported in Table 2. Up to 40 mg/kg of animal weight of synthetic alliin, the specific principle of garlic, or garlic extract (.02–.05 ml/injection) did not have any significant tumor-inhibiting properties. Necrosis and the collapse of veins followed repeated intravenous injection of allicin.

Six inhibition tests with *S. cerevisiae var.*, *S. ellipsoidue*, *S. cerevisiae 1741*, and *S. carlsbergensis*, each with the synthetic alliin, resulted in no inhibi-

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tion of any yeast parent or respiratory mutant derived from them with a concentration of 86 μg. of alliin per disc. A concentration of 86 μg. of the enzymatically prepared allicin per disc resulted in inhibition of all the yeast parents; whereas, in the six mutants derived from them, two were not inhibited, one was inhibited to the same extent as the parent yeast, and three were inhibited to a greater extent than the parent.

DISCUSSION

Although allicin is a potent bactericidal agent (2) and has been shown to preferentially inhibit sulfhydryl enzymes without affecting many others (11), it has not been possible to demonstrate a consistent and significant inhibition of transplantable mouse tumors either by the injection of alliin, which in the presence of a specific enzyme might split in the animal body to allicin, or by the enzymatically produced allicin. The *in vitro* incubation of ascites with allicin prior to intraperitoneal injection into mice has tumor-inhibiting effects which might be attributable to either a combination of allicin with cysteine or the oxidation of sulfhydryl to disulfide in —SH-containing enzymes by the labile oxygen (2). Weisberger and Pensky (8), using analogs of allicin, concluded that these analogs might have tumor-inhibiting effects.

TABLE 1

THE INHIBITION OF ASCITES TUMOR FORMATION BY PREINCUBATION WITH ALLICIN

5×10^6 cells/mouse

Tumor	No. mice	Conc. of allicin (μg/0.2 ml suspension/mouse)	Survival time (days)
Ehrlich-2	5	810	90*
"	5	81	90*
"	5	16	60*†
"	5	8	25
"	5	4	22
"	5	2	20
	26		20
Sarcoma 180	10	810	90*
"	10	81	90*
"	10		15

* Killed. † One with solid tumor.

TABLE 2

EFFECT OF ALLICIN ADMINISTRATION* UPON TUMORS

Tumor system	Results†	No. animals	Deaths	Dosage (μg/mouse/day)	Total no. injections	Animal wt. changes‡	90 per cent survival mean (days) Exp.	Control	Per cent inhibition
Ehrlich-2 ascites	—	5	0	600	7		27	24	
L1210 ascites	±	10	0	645	7		15	12	
"	±	6	0	86	7		14	11	
S-180 ascites	±	7	0	860	1§		25	18	
"	±	9	4	800	6		23	19	
"	—	9	0	700	7		22	19	
"	—	7	0	345	7		20	19	
"	—	6	0	172	7#		18	19	
"	—	7	0	258	7#		20	19	
"	—	9	0	700	7§		19	19	
S-180	—	7	0	860	1	−0.1/+0.7			−24
"	—	7	0	700	7	−2 /+0.3			14
"	±	7	0	43	7	−1.2/−0.6			34
"	—	7	0	86	7	−1.1/−0.6			22
"	—	8	0	700	7§	−1.1/−0.2			13
"	—	8	0	700	7#	−1.2/−1.2			7
"	—	7	0	516	7‖	−1.3/−0.7			−10
C-755	±	8	2	700	14	−1.5/+1.6			55

* I.P. unless otherwise noted.

† In the ascites tumors, ± indicates 20–50 per cent increase in survival time of experimental group; — indicates less than 20 per cent inhibition. Per cent inhibition of S-180 was determined by mean diameter, and for C-755 it was determined by mean tumor wt. A negative number indicates the extent to which tumors of the treated group exceeded the control group.

‡ Av change of treated/av change of control.

§ I.V. # Orally. ‖ Subcutaneously.

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Although our results with allicin are similar to their results with analogs, the absence of significant and consistent inhibition in tumor-bearing mice leads to the conclusion that alliin and allicin do not appear promising for cancer chemotherapy.

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THE EFFECT OF INCORPORATION OF GARLIC IN THE DIET ON THE INTESTINAL MICROFLORA OF RATS

V. SUBRAHMANYAN, K. KRISHNAMURTHY, V. SREENIVASAMURTHY AND M. SWAMINATHAN

From the Food Technological Research Institute, Mysore

(Received for publication, April 28, 1958)

The efficacy of several spices and condiments in curing certain intestinal disorders has been known in the Indian system of medicine (1, 2). In recent years considerable attention has been paid in finding out the active principles present in them and their mode of action (3—6). Subrahmanyan *et al.* (7) have studied the effect (*in vitro*) of some of the more commonly used spices on the intestinal bacteria in health and disease, and reported that garlic was the most potent in inhibiting the growth of some of them. The therapeutic activity of garlic is due to a chemical compound known as allicin which has antibiotic properties (8). Several workers have reported that allicin inhibits *in vitro* the growth of a number of micro-organisms (8, 9). In another communication from this laboratory (10), it has been reported that incorporation of large amounts of pulses, ragi (*Eleusine coracana*) or milk in a poor rice diet, produced a marked increase in the coliform and anaerobic counts in the caeca of albino rats. It was therefore considered of interest to study whether garlic could prevent the increase of the intestinal microflora when such diets are used.

EXPERIMENTAL

Preparation of garlic extract.—According to Ayurvedic practice, a daily dose of 10-15 g. of garlic is usually given to cure different intestinal disorders (1). Garlic bulbs were macerated in 4 times its weight of water and filtered through cloth. Each rat received 2·5 cc. of the extract equivalent to 0·5 g. garlic daily.

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Influence of administration of garlic for varying periods on the intestinal microflora.—Preliminary studies were conducted to find out the minimum period of administration of garlic required to reduce the count of caecal micro-organisms in adult rats fed on stock diet and synthetic diet. Adult male rats weighing 150-200 g. were divided into four groups. The first two groups were fed synthetic diet and the other two groups were fed stock diet for a period of three weeks. The composition of the experimental diets is given in Table I. At the end of three

TABLE I

Composition of the experimental diets

Stock diet *		Synthetic diet		Poor rice diet (PRD) with supplements (%)		
Components	%	Components	%	Components	PRD + Red gram dhal	†PRD + milk
Ragi (*Eleusine coracana*)	20·8	Starch ..	69	Rice (milled) ..	23·5	78·5
Wheat (whole) ..	20·8	Casein ..	12	Red gram dahl (*Cajanus cajan*) ..	60·0	5·0
Groundnut cake ..	3·5	Salt mixture (Osborne & Mendel)	4	Common salt ..	0·3	0·3
Bengal gram (*Cicer arietinum*) ..	3·5	Vitaminised starch **	4	Skim milk powder	0·9	0·9
Skim milk powder ..	10·5	Groundnut oil ..	10	Groundnut oil ..	5·0	5·0
Common salt ..	1·0	Shark liver oil ..	1	Leafy vegetables ..	2·1	2·1
Calcium carbonate ..	0·4			Non-leafy vegetables	8·2	8·2
Groundnut oil ..	3·5	** Supplies the daily requirements of all the B-group vitamins.		† The diet (12 g.) was cooked with 4 times its weight of water and mixed with 10 cc. of skim milk and given daily to each rat.		
Shark liver oil ..	1·0					
Lucerne ..	14·0					
Amaranthus gangeticus leaves ..	14·0					
Carrot ..	3·5					
Liver ..	3·5					

* Twelve g. of the diet were cooked in 4 times its weight of water and given daily to each rat.

weeks, 2·5 cc. of garlic extract (equivalent to 0·5 g. garlic) per rat per day were administered through a stomach tube to one group of rats on each diet, one hour after feeding. The analysis of the caecal microflora in the control and experimental rats was carried out for coliforms, total count and anaerobes, according to the methods described in an earlier paper (11). The results are given in Tables II and III.

TABLE II

Effect of garlic on the caecal flora of rats fed synthetic diet (2·5 cc. of garlic extract was given per rat per day through a stomach tube)

No. of days of feeding rats with garlic	Weight of dry caecal contents (mg.)	Coliforms $\times 10^3$		Total count $\times 10^4$		Anaerobes $\times 10^6$	
		per mg. dry wt.	per caecum	per mg. dry wt.	per caecum	per mg. dry wt.	per caecum
0	194	12	1,300	182	36,100	8	1,500
1	336	1	300	70	23,100	3	900
3	232	1	200	85	21,000	1	200
5	236	1	200	80	19,000	3	700

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TABLE III

Effect of garlic on the caecal flora of rats fed stock diet (2·5 cc. of garlic extract was given per rat per day through a stomach tube)

No. of days of feeding rats with garlic	Weight of dry caecal contents (mg.)	Coliforms × 10^3		Total count × 10^4		Anaerobes × 10^6	
		per mg. dry wt.	per caecum	per mg. dry wt.	per caecum	per mg. dry wt.	per caecum
0	796	17	13,000	88	70,000	5	4,000
1	524	24	12,800	115	60,200	3	1,600
3	870	18	15,900	118	104,000	2	1,740
5	843	3	2,900	15	12,600	1	843
8	753	2	1,510	18	13,590	1	753

The effect of incorporation of garlic in the diet on the caecal microflora of rats fed poor rice diet supplemented with milk and red gram dhal respectively.—Four groups of adult male rats weighing 150-200 g. were used. The rats in the first two groups were fed poor rice diet in which 75% of the rice was replaced by red gram dhal. The rats in the other two groups were fed poor rice diet supplemented with 10 cc. of skim milk per rat per day. The animals were given the diets for three weeks, at the end of which 2·5 cc. of garlic extract (equivalent to 0·5 g. garlic) was given per rat per day to one group of rats on each diet. The garlic extract was mixed in the daily ration and fed to the rats for a period of five days. At the end of the experiment, the rats (both the control and experimental groups) were sacrificed and the analysis of caecal microflora for coliforms, total count and anaerobes was carried out. The results are presented in Table IV.

TABLE IV

Effect of garlic in the diet on the caecal flora of rats fed rice diet supplemented with milk or red gram dhal

Diet	Weight of dry caecal contents (mg.)	Coliforms × 10^3		Total count × 10^4		Anaerobes × 10^6	
		per mg. dry wt.	per caecum	per mg. dry wt.	per caecum	per mg. dry wt.	per caecum
Poor rice diet ..	320	3·0	900	16	5,000	1·0	320
Poor rice diet + milk ..	300	15·0	4,310	63	19,000	13·0	3,800
Poor rice diet+milk+garlic* ..	373	1·8	720	9	3,400	1·8	650
Poor rice diet+red gram dhal ..	830	1·8	1,430	28·0	23,630	22·0	18,300
Poor rice diet+red gram dhal+garlic* ..	690	1·2	740	5·5	4,190	6·5	4,800

* 2·5 cc. of garlic extract (=0·5 g. of fresh garlic) was given per rat per day along with the daily ration, for five days.

Results and Discussion

It may be seen from Table II that the caecal contents of rats receiving stock diet were more bulky and had higher numbers of coliforms, total count and anaerobes than those of rats receiving synthetic diet. This may be because stock diet is rich in roughage and indigestible matter, and favours the multiplication of various organisms in the caecum.

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The results in Tables II and III show that in the case of rats receiving synthetic diet, administration of garlic even for one day reduced the number of coliform, total aerobic and anaerobic organisms considerably. On the other hand, in the case of rats receiving stock diet, the reduction in the caecal flora was noticed only after administering garlic for five days.

Data regarding the count of coliforms, total aerobic and anaerobic organisms in the caeca of rats receiving poor rice diet are also given in Table IV. They show that the caeca of rats receiving poor rice diet supplemented with red gram dhal had very high anaerobic count and those of animals receiving milk supplement had high coliform, total aerobic and anaerobic counts. The administration of garlic along with the diet for five days brought down considerably the count of the above organisms.

Summary

1. Studies on the effect of incorporation of garlic in the diet on the caecal flora of rats showed that in the case of rats receiving synthetic diet, administration of garlic even for one day reduced the caecal flora considerably, but in the case of rats receiving stock diet the reduction in the caecal flora was noticed only after the administration of garlic for five days.

2. The abnormal increase in the caecal flora of rats receiving poor rice diet supplemented with red gram dhal or milk was reduced considerably by the administration of garlic for five days.

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The Treatment of Gapeworm Disease.

By PHYLLIS A. CLAPHAM, B.Sc., Ph.D.

(Lawrence Research Student of the Royal Society)

(From the Institute of Agricultural Parasitology, St. Albans, London School of Hygiene and Tropical Medicine)

IN a note in the British Medical Journal for February 3rd, 1934, there is mention of the anthelmintic action of garlic oil. One paragraph says that good results have been obtained in the treatment of threadworm in children while a second is more specific and quotes the case of a woman who was cured of *Ascaris lumbricoides* in 36 hours. She passed a male, 13¾ inches long, a female and a bunch of newly hatched young.

Dr. R. T. Leiper brought this to my notice and suggested that as garlic oil is excreted by the lungs it might be effective in the treatment of gapeworm disease. Messrs. Allen and Hanburys Ltd. very kindly supplied me with natural oil and with the synthetic product, allyl sulphide. I am much obliged to them for this favour.

Many attempts have been made in the past to find a cure for gapeworm disease but these have met with but little success as, being sidetracked in the trachea, the worms are somewhat inaccessible. Treatments by drugs via the gut have never met with enough success to warrant their use. Fumigation and inhalation have been tried: the principle being that the inhaled fumes would either irritate the worms until they released their hold or would stupify them so that, in either case, they could be coughed up by the host. Intratracheal injection of a dilute (5–8 per cent.) solution of salicylic acid by means of a small syringe has been recommended but the most popular method of treatment seems to have been the mechanical removal of the worms by means of twisted horse hairs or of partially stripped feathers. This method is not entirely successful and has the disadvantage of being tedious to the operator and irritating to the chicken. In many cases of disease, however, it would appear that the custom has been to let the chicks die. Up to the present prophylaxis has been the more useful line of work.

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With garlic oil the present writer has had some considerable success. Three methods of treatment have been used. The first was the anointing of the feathers of the head and neck so that the birds were compelled to inhale the fumes. No success was realised by this method and by reason of its odour, it had the great disadvantage of being very unpleasant to both human beings and birds. The second method was to mix the oil with the food or water but this was not successful either. One minim of oil per bird was mixed with the day's ration of food but the birds refused to eat, even though this food was left there in front of them without change for some time until it had become stale. Even when the birds were compelled to eat a quantity, by means of forcible feeding, yet cure did not occur. There are two possible causes of this. The birds probably did not get a sufficient concentration of oil passing through the lungs and up the trachea to kill the parasites, or it may be that the presence of food may alter the absorption or reaction of the drug.

That the birds found this concentrated oil very unpleasant was obvious by the fact that when mixed with the food or water, they refused to eat or drink it and it was impossible to make them take it by themselves. It was necessary therefore to administer it by means of a fine pipette which could be inserted well back into the throat. This led to the third method of treatment, which proved highly satisfactory.

Pipettes were drawn out finely so that the oil could be administered in minims. When smaller doses were required, a solution was made up in linseed oil. The usual percentage of oil in these solutions was either 50% or 33⅓%.

The birds used in these experiments had been artificially infected with the parasite using the intermediate host *Eiseuia foetida*. The method in general use was to divide the birds into two groups, one of which remained untreated as a control. In the other group the faeces were examined before and after treatment and after the oil had been administered search was made in the cage for any worms that might have been expelled. When the treatment was over, the birds were killed and the tracheae in the two groups compared. As will be seen later it was almost impossible to discover the number of worms expelled by the chickens by searching in the cage as the drug caused them to rupture. Comparisons with the control group were not satisfactory either as it was not possible to estimate the degree of infection of the intermediate host. Hence in later experiments this control group was dropped and more reliance was placed on the

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disappearance of clinical symptoms and of eggs in the faeces. Furthermore when the birds were killed within a few hours after the last treatment with the drug, it was often possible to see a small inflamed papilla, with a petechial haemorrhage, indicating the site of adhesion of a worm.

Altogether three sets of experiments were carried out, involving about 50 birds. The dosage ranged from ⅓ minim to 3 minims of pure oil. It may be remarked here that no difference was observed between the actions of the natural and the synthetic products. Hence the synthetic product, allyl sulphide, is preferable as this seems to have the slightly less pungent odour and is considerably cheaper to buy.

The chicks used were between the ages of 13 and 22 days and the most successful treatment was as follows:—⅓ minim of the drug in linseed oil solution was given three times a day orally by pipette. Plenty of water was allowed all day and food was placed before them about 5·30 p.m. and left until morning. A small feed was given about 2 p.m. The times and method of dosing is given in a table, together with a representative sample of results.

It was found that 2 minims per day divided into three doses was the limit that an infected chick could stand: even then there was a mortality of over 6 per cent. This, given in a single dose, killed practically all the infected birds though healthy birds were not quite so severely affected. Less than ½ minim of oil per day was not satisfactory as an anthelmintic.

The effect of the garlic oil is very marked and is quickly seen. During the first day heavily infected birds cease gaping and only the sneezing is seen. This latter symptom gradually moderates and about three days after treatment, has subsided and the birds seem cured. Three days was usually enough for the birds to cough up the parasite which had been released by the oil.

The action of the drug is interesting. The parasites are markedly affected and by some reaction the body wall is weakened and ruptured allowing the genitalia to escape and be exposed. The coughing of the host caused the fine tubes to break and to be gradually brought up. Because of this reaction it was impossible to recover gapeworms from the floor of the cage during treatment, though portions were sometimes observed. That the action of garlic oil is very powerful was seen in the fact also that all the eggs recovered were sterilised. On several occasions, attempts were made to culture them but no development ever occurred. The contents were dark and opaque and within a day or two, decomposition

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with the deposition of oil granules was apparent. It was rare to find eggs that had escaped this action of the oil. This seems to the writer to be an observation of importance, as on occasions, a number of parasites were not dislodged as a result of treatment but the eggs were always non-viable and therefore would no longer be a source of danger to other birds.

It was, however, realised that individual treatment of chickens would probably prove to be a tedious business and not always practical during an epidemic. For this reason attempts were made to administer the oil in food or water. These have already been described.

Summarising these results we may say that garlic oil has a very marked effect on *S. trachea*. It causes the worm to rupture, thus killing the parasites when the birds may cough them up. The eggs which the gapeworms contain are sterilised even though the worms may be retained within the body for a longer or shorter period after treatment.

Carbon tetrachloride was another drug used against *S. trachea*. In a letter to Nature 1931, p. 585, Atkins mentions that he has used this drug against gapes successfully. In a private letter to me he kindly gave me his technique and his results. The treatment seems to be quite useless, however, for chicks up to 14 days old.

The CCl_4 was administered by means of a pipette as a solution in thick medicinal paraffin. Two solutions—containing respectively 5 and 10 per cent. of the drug were used. It was given orally. Treatment was never hurried: it usually took about 5 minutes to give the dose to the chicken. By this means the substance was made to hang about the throat and the chick almost gargled during this time, so that the substance reached the trachea by two routes—by inhalation during the actual administration and later by excretion from the lungs after absorption from the blood stream.

Some success is to be recorded as a result of treatment with this substance. It was found extremely useful for chickens that were suffering severely and in the last stages of the disease. In no case, however, was a complete cure effected but considerable relief was often apparent. The severe symptoms disappear rapidly and though living parasites were always found at post-mortem yet they were never in sufficient numbers to cause distress to the bird.

A 5 per cent. solution was found to be the most effective and the most satisfactory dose was 1 cc. twice a day. The first was usually given about 9.30 a.m. and the second at 5.30 p.m. Considerable alleviation was

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Chick.	Age in days	Treatment				Died	Remarks
		Substance	Date	Time	Quantity		
18	21	Natural oil	14.3.34	11 a.m.	minims 1	Killed 27.3.34	Gaping ceased but eggs still passed in faeces. 1 pair gapes retained.
6	21	Natural oil	14.3.34	11 a.m.	2	14.3.34 2 p.m.	6 pairs of gapes—dead and ruptured.
10	21	Natural oil	14.3.34	11 a.m.	3	14.3.34 1.30 p.m.	5 pairs of gapes—dead and ruptured.
14	21	Synthetic oil	14.3.34	11 a.m.	1	Killed 16.3.34	5 pairs of gapes—dead and ruptured.
12	21	Synthetic oil	14.3.34	11 a.m.	2	Killed 15.3.34	4 pairs of gapes—as above
13	21	Synthetic oil	14.3.34	11 a.m.	3	14.3.34 2 p.m.	21 pairs of gapes, all much broken up.
15	19	Natural oil	27.7.34 28.7.34	11 a.m. 4 p.m. 9 a.m. 1 p.m. 4 p.m.	½ ½ ½ ½ ½	Killed 2.8.34	All symptoms disappeared by 1.8.34. No eggs found in the faeces and no worms were in trachea.
3	13	Synthetic oil	21.6.34 22.6.34	11 a.m. 4 p.m. 9 a.m. 1 p.m. 4 p.m.	½ ½ ½ ½ ½	Killed 4.7.34	Few infertile eggs in the faeces. 1 pair of gapes in trachea and some fragments of female genitalia, containing infertile eggs. 5 papillae in trachea, indicating position of other gapes.
7	13	Synthetic oil	21.6.34 22.6.34	11 a.m. 4 p.m. 9 a.m. 1 p.m. 4 p.m.	½ ½ ½ ½ ½	Killed 4.7.34	Eggs in faeces. 1 pair of gapes, brown but still alive. All eggs were infertile.
19	13	Natural oil	21.6.34 22.6.34	11 a.m. 4 p.m. 9 a.m. 1 p.m. 4 p.m.	½ ½ ½ ½ ½	Killed 27.6.34	All symptoms had ceased. Some broken genital tubes in trachea and 1 male. 7 papillae on wall.
26	13	Synthetic oil	as above	as above		Killed 26.6.34	No gaping but some sneezing remained. A mass of genital tubes was lodged near top of trachea in mucus. All eggs were sterile.

Table 1. Indicating the results obtained from the use of garlic oil in a representative sample of birds.

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observed after a single day's treatment and the birds continued to improve after another day's treatment though fertile eggs still appeared in the faeces. Further treatment never effected a cure. In two cases, very heavily affected birds were available for experiment. Both these birds were very sick and showed every appearance of an early death. They were 15 days old at the time: the infection being of 6 days duration. They were each given 1 cc. of the 5 per cent. solution 4 times a day, at 9.30 a.m., noon, 3.30 p.m. and 6 p.m. In the evening they were much stronger and could stand instead of lying collapsed on the floor of the cage. Next day a considerable improvement was obvious. They were much stronger and were feeding though they were still gaping. Treatment was discontinued for 36 hours owing to the toxic action of the drug on the liver. Then they were given another dose which was the last. The faeces were examined for 4 days after this and fertile eggs were found continuously. They were killed and at post-mortem one showed a single pair of gapes and the other 3 pairs in the trachea. The mucosa of the trachea was inflamed and from the presence of 5 small papillae one can assume that 5 pairs of gapeworms had been removed in each bird by means of this treatment. When the birds were killed, they were still sneezing though they had not been observed to "gape" for 2 days.

Certain infected chicks were dosed with either 1 cc. or 2 ccs. twice daily of a 10 per cent. solution of this substance but less certain results were obtained. The reason for this was not apparent until a phrase in Chopra and Chandler's "Anthelmintics and their uses" p. 112, was noticed. They say ". . . When given in large doses most of the drug appears to pass out in the faeces, partly in combination with fats and fatty acids." It would seem therefore that 1 or 2 ccs. of a 10 per cent. solution was a too heavy dose for chicks of that age. None of them died, however, as a result of the toxic effects of the susbtance in this case.

The action of CCl_4 is very rapid. It is absorbed and excreted by the lungs within a few minutes after administration. For this reason it may be particularly useful when the patient is in the last stages of the disease as giving quick relief. But its marked toxic reaction must not be forgotten.

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Summary

In clinical trials of piperazine hydrate, gentian violet, and diphenan against oxyuriasis in 136 children, piperazine hydrate syrup and gentian violet showed marked oxyuricidal properties, while diphenan was inactive.

Owing to lack of toxicity and side-effects, to ease of administration, and to its excellent therapeutic properties, piperazine hydrate syrup at a dose level of 50–75 mg./kg. per day is considered to be the drug of choice for oxyuriasis in children. Of 31 children treated at levels greater than 50 mg./kg. daily 97% were cured.

The use of suppositories is unnecessary.

Spontaneous cure may be expected in 19% of cases of oxyuriasis.

The facilities provided by Dr. Philip R. Evans in the Department of Child Health at Guy's Hospital and the Evelina Hospital, London, are gratefully acknowledged. The guidance and advice of Dr. R. C. MacKeith, Guy's Hospital, are deeply appreciated. Without the co-operation of the medical and nursing staffs at these two hospitals this work could not have been completed, and to them our greatest thanks are extended.

The piperazine hydrate used in these trials was provided by Messrs. Burroughs Wellcome and Co. as "antepar" elixir.

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EXPERIMENTAL CHEMOTHERAPY OF OXYURIASIS

BY

O. D. STANDEN, M.Sc.

From the Wellcome Laboratories of Tropical Medicine, London

The two drugs most commonly used in the treatment of oxyuriasis are gentian violet and diphenan. From time to time claims of efficacy in clearing threadworms have been made for other substances, notably aluminium 8-hydroxyquinoline ("nyxolan"), N-*iso*amylcarbaminic acid-3-methyl-6-*iso*propyl phenyl ester ("egressin"), and garlic preparations. Owing to the length of the life cycle and the possibilities of auto-infection, properly controlled clinical trials against *Enterobius* (*Oxyuris*) *vermicularis* are extremely complicated. The eight weeks or so required for diagnosis, treatment, and follow-up is longer than children usually remain in hospital, and any trials started under the control and supervision of the medical and nursing staff must be continued with the co-operation of the parents. The difficulties inherent in such an arrangement probably account for the relatively few occasions upon which adequate clinical trials have been made with potential oxyuricides. Obviously some laboratory method of screening drugs of this kind is desirable, so that these cumbersome clinical trials may be initiated only when justified by experimental evidence.

As no laboratory animal has been found that will act as host to *E. vermicularis* the oxyurids of rodents have been employed for chemotherapeutic trials. The laboratory evaluation of egressin was carried out using *Passalurus ambiguus* in the rabbit (Eichholtz and Hotovy, 1950); *Aspiculuris tetraptera*, oxyurid of the mouse, was used for study of the oxyuricidal properties of antibiotics (Wells, 1951a, 1951b, 1952a, 1952b); *Syphacia obvelata* in the mouse was used by Chan (1952) for screening a number of potentially active substances, while Turpin *et al.* (1952) tested piperazine against both *Aspiculuris* and *Syphacia*. Mouriquand *et al.* (1951) used piperazine hydrate against *A. tetraptera* in the mouse.

A. tetraptera occurs naturally in the caecum and rectum in many laboratory strains of white mice. For the experimental work here described it was decided to use such natural infections with this parasite and to carry out a series of tests on substances of reputed activity against human threadworms. Tests on certain closely related substances were also to be included. By this means it was hoped to determine whether tests against *A. tetraptera* in the mouse would provide valid indications of activity against *E. vermicularis* in man and to demonstrate substances worthy of clinical trials.

Material and Method

White mice 11–12 weeks old showing ova of *Aspiculuris* in the faeces were segregated and used for drug-testing. Necropsy of untreated mice showed a range of 2–120 worms per mouse. Because of the relatively small number (10–20) of mice that could be used in each test it was considered that this range in numbers of worms was too large to warrant valid statistical deductions to be drawn on the basis of reduction in worm numbers following treatment. Therefore the degree of activity of a drug was estimated in relation to the dose required to clear the worms from all or a proportion of the mice treated. The drugs were given orally by stomach tube as a solution or suspension in water or liquid paraffin. The animals were dosed either once or twice daily for five consecutive days. According to the availability of infected mice, 10–20 animals were used for each test. Faecal examinations were made at six days and necropsy at 12 days after completion of treatment. In some instances necropsy was carried out at six days after treatment without prior faecal examination.

To facilitate worm counts the rectum and caecum of each mouse were removed at necropsy and placed in Bouin's fixative. Material fixed in this way could be stored and examined when convenient. At examination this portion of the gut was slit open under water and the contents were removed gently with a camel-hair brush. The inside of the examination dish was painted black, and the worms, now stained bright yellow, were easily seen and counted against this background.

Results

Table I shows the relative efficiency of a number of reputed oxyuricides in clearing mice of *Aspiculuris*. None of the drugs used, except perhaps oil of garlic, was particularly toxic* at the dosages employed, and only two, gentian violet and piperazine hydrate, produced any marked worm clearance. In seven other instances partial clearances were obtained, but four of these (Nos. 4, 6, 10, and 13)

* All mice on test had been exposed to infection with *Schistosoma mansoni*. A proportion of the mouse mortality would result from this.

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TABLE I.—*Showing the Relative Efficiency of Several Reputed Oxyuricides and Some Related Compounds in Clearing Mice of Naturally Acquired Threadworm (Aspiculuris tetraptera) Infections*

No.	Drug	Oral Dose (mg./kg.)	No. of Doses Daily	Total Dose (mg./kg.)	Mice Surviving at Necropsy	Days After Treatment	% Mice Cleared of Worms	Average No. of Worms per Mouse	Range in No. of Worms	Drug Efficiency Rating
1	Gentian violet	90	1	450	16/20	12	80	7	2–11	+++
2	Piperazine hydrate	400	1	2,000	13/13	12	100	0	—	++++
3	N-carbethoxypiperazine hydrochloride	400	1	2,000	12/13	12	0	44	8–183	—
4	1-Diethylcarbamyl-4-methylpiperazine citrate ("hanocide," "hetrazan")									
	.. .. " syrup	500	1	2,500	9/10	12	10	24	2–77	—
	.. .. " powder	500	1	2,500	9/12	12	0	37	4–152	—
5	N-isoamylcarbaminic acid-3-methyl-6-isopropyl phenyl ester ("egressin")	200	2	2,000	9/12	6	0	68	11–158	—
6	Aluminium 8-hydroxyquinoline ("nyxolan") syrup	60	2	600	10/10	12	10	37	2–94	—
7	Diphenan	700	1	3,500	18/20	12	0	101	18–154	—
8	Quinoxaline	150	2	1,500	11/12	6	0	94	37–187	—
9	Mepacrine	50	2	500	12/12	6	25	29	3–60	+
10	Phenothiazine	100	1	500	12/13	12	8	62	15–114	—
11	Santonin	200	1	1,000	9/10	12	20	38	3–107	±
12	Hexylresorcinol	20	1	100	9/12	12	0	67	2–125	—
		10	1	50	12/13	12	40	59	16–163	+
13	Dried garlic ("a' isatin")	200	2	2,000	8/10	12	10	83	53–165	—
14	Oil of garlic	½ minim per kg.	2	2½ minims per kg.	6/10	12	0	45	5–121	—
15	Allyl sulphide	"	2	"	8/10	12	0	61	2–121	—
16	Liquid paraffin	10 ml. per kg.	2	100 ml. per kg.	5/5	12	0	56	20–72	—

TABLE II.—*Showing the Graded Dosage Effect of Gentian Violet and Piperazine Hydrate in Clearance of Aspiculuris tetraptera from Naturally Infected Mice*

Drug	Oral Dose (mg./kg.)	No. Doses Daily	Total Dose (mg./kg.)	Mice Surviving at Necropsy	Days After Treatment	% Mice Cleared of Worms	Average No. of Worms per Mouse	Range in No. of Worms	Drug Efficiency Rating
Gentian violet	100	1	500	5/10	12	100	0	—	++++
	90	1	450	16/20	12	80	7·0	2–11	+++
	50	1	250	10/10	12	100	0	—	++++
	25	1	125	10/10	12	0	31·0	2–61	—
Piperazine hydrate ..	400	1	2,000	13/13	12	100	0	—	++++
	200	1	1,000	10/13	12	90	0·1	0–1	+++
	100	1	500	13/13	12	62	3·0	1–8	++
	50	1	250	12/13	12	31	40·0	2–212	+

were of 10% or less, and can be ignored. Other clearances—hexylresorcinol (40%), mepacrine (25%), and santonin (20%)—were not convincing. In the case of hexylresorcinol the 40% clearance was obtained with 10 mg./kg. unit dose, but no clearance resulted when the dose was doubled. Obviously some spontaneous clearance may occur, and unless reasonably uniform results are produced in repeated tests isolated successes must be disregarded.

The clearance rates obtained with gentian violet and piperazine were further investigated. The results of these further tests, together with the original ones, are given in Table II. It will be seen that gentian violet gave a ++++ rate at 50 mg./kg. unit dose, but failed to clear at 25 mg./kg. Piperazine hydrate gave a good response at 400 and 200 mg./kg. unit dose, and at most dose levels the numbers of worms left were very small. In the piperazine 50 mg./kg. group one mouse had 212 worms, whilst the average for the remaining seven positives was only 15. At the 200 mg./kg. level only one mouse remained infected, and this had only one worm, whilst the scanty worm burden remaining at the 100 mg./kg. dose level is perhaps additional evidence of drug activity.

It may be concluded that, in terms of dose/weight ratio, gentian violet is rather more efficient in clearing mice of *A. tetraptera* than is piperazine hydrate, but, nevertheless, piperazine is highly promising and is worthy of clinical trial. The rate at which the intestinal contents move in the mouse is very high compared with that in man, and it is conceivable that in the human subject the piperazine may be absorbed or destroyed before it reaches the worms.

Since this work was completed, clinical trials of piperazine hydrate, gentian violet, and diphenan have been concluded (White and Standen, 1953). The activity of piperazine hydrate and gentian violet against *Aspiculuris* in the mouse is confirmed against *Enterobius vermicularis* in children, whilst diphenan, inactive against *Aspiculuris*, was similarly shown to be of no value in the treatment of human oxyuriasis. The comparative evidence so obtained justifies the use of *Aspiculuris* in a laboratory screen against threadworm infestation.

Summary

Aspiculuris tetraptera, threadworm of the mouse, was used to test the efficacy of reputed oxyuricides. Of these, only gentian violet and piperazine hydrate cleared the mice of worms.

Drug-testing against *Aspiculuris tetraptera* in the mouse is of considerable value in establishing the efficiency of oxyuricides prior to clinical trial.

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Regulations which came into effect on September 1 control the manufacture of aureomycin. When it is intended for parenteral injection it may not be manufactured for sale except under licence and in conformity with prescribed conditions as to strength, quality, and purity. The regulations are entitled the Therapeutic Substances Amendment Regulations, 1953.

Comptes Rendus (Doklady) de l'Académie des Sciences de l'URSS
1943. Volume XXXVIII, № 4

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EXPERIMENTAL BIOLOGY

PHYTONCIDES AND THEIR BACTERICIDAL PROPERTIES

By A. FILATOVA

(Communicated by L. A. Orbeli, Member of the Academy, 12.XI.1943)

The name of «phytoncides» was given by B. Tokin to the volatile substances he had discovered. These substances whose chemical nature is still uncertain are emitted by plants in the course of their life-activity and are endowed with bacterio- and protistocidal properties.

The present author found in collaboration with Dr. A. Tebjakina (1932)(1) that phytoncides of *Allium cepa* L. and *Allium sativum* L. are apt to kill *Bacillus coli*, *B. proteus*, *B. typhi abd.* and *B. staphylococcus*. Even at that state of investigation the discovery of the new bactericides of vegetable origin appeared promising from the standpoint of practical medicine. In so far as Tokin's discovery was of purely biological interest, too, the present author applied himself during several years to thoroughly investigate the bactericidal properties of phytoncides. This note contains a part of the experimental data secured by him.

Out of more than 150 vegetable species investigated by Tokin and his co-workers I dealt largely with onion and garlic.

In the first line, I tested the effect of *Allium cepa* L. upon *B. staphylococcus* and *B. sarcina*. The technique of the experiments was the following. Two drops of a milliard suspension of *Staphylococcus albus* were put on the bottom of a Petri dish; they were added with 10—15 drops of juice freshly squeezed out from an onion, and covered with agar-agar. An experimental culture prepared in this way was placed into a thermostat at a temperature of 36—38° and observed daily. As a control served a similar culture without addition of onion juice. Similar experiments were carried out also with *B. sarcina*.

Out of the 29 tests with staphylococcus 27 gave positive results: in a number of cases the experimental culture showed but a poor growth as compared to the opulent growth in the control, occasionally there was total sterility.

Out of the 10 experiments with sarcina 6 gave the result that should have been anticipated.

Subsequent experiments were run with the purpose to make clear whether the phytoncides have a sterilizing effect upon staphylococci and sarcina, when applied as vapour.

Two drops of a milliard suspension of bacteria were put on the bottom of a Petri dish, added with agar-agar, after which the dish was closed. There was taken another Petri dish with some freshly mashed onion on the bottom. The bacterial culture prepared in the first dish was treated with phytoncide vapour during ten minutes. With this aim in view the former was placed with the bottom upwards over the latter dish containing the onion paste. As control served an identical growth which had not been subjected to a treatment with phytoncides.

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The same bacteria were also tested for the effect of garlic phytoncides, the procedure being essentially the same.

Along with experiments of this kind there were also arranged tests with allyl-mustard oil and water extract of *Pinus pumila*. In the first set, a small amount of table mustard was stirred in some warm water. The experiments showed these substances to possess an exceedingly high bactericidal effect, too. In agreement with the statement advanced by B. Tokin, however, I am not inclined to identify the chemical nature of the phytoncides with that of essential oils of plants.

The results of the experiments are summarized in Table 1.

Table 1

Effect of Phytoncides upon Staphyllococci

Treatment with juice			Treatment with vapour		
Plant	Number of tests	% of cases with positive results	Plant	Number of tests	% of cases with positive results
Allium cepa . . .	29	93	*Allium cepa* . . .	40	60
Allium sativum .	8	100	*Allium sativum* .	11	55
Pinus pumila . .	17	94	Mustard	20	70

Effect of Phytoncides upon *B. sarcina*

Treatment with juice			Treatment with vapour		
Plant	Number of tests	% of cases with positive results	Plant	Number of tests	% of cases with positive results
Allium cepa . . .	10	60	*Allium sativum* .	11	73
Allium sativum .	5	100			
Pinus pumila . .	9	100	Mustard	21	71

The question arises, whether the phytoncides act upon the nutrient medium making it unfit for bacterial growth, or whether their effect is a direct one upon the bacteria?

Experiments arranged with the purpose to settle this point have shown that they affect bacteria directly.

Experiments carried out by other authors go to confirm the results obtained by us.

Particularly illustrative is the picture obtained by Tokin and Nelinbova in their experiments on the effect of phytoncides upon *Bacillus tuberculosis* of strain BCG, since the method applied in that case was a 6-minute direct treatment of the bacteria with phytoncide vapour, previous to sowing them in the nutrient medium.

Not all our experiments offered a picture of complete sterility, after the bacterial culture had been subjected to phytoncide treatment; as a matter of fact, the results were rather variegated.

There were cases when the ultimate results of growth proved identical both for the experimental and control culture.

It is no difficult matter to explain this circumstance.

Let us suppose that not all the bacteria, but only part of them have been killed by the action of the phytoncides. If the conditions happen to be favourable for the growth of bacteria, the experimental culture may overreach the control culture, if the latter is in the process of depression.

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In such cases the experimentalist is bound to investigate the dynamics of growth of a given culture.

The following circumstance should also be taken into account.

First, as a general rule, the phytoncides produce a powerful sterilizing effect only at the moment they are emitted. Sure enough, because of the technical procedure of the experiments, the bacteria could not always be acted upon with the vapour emitted by the phytoncides immediately, and this is why the effect obtained may vary from experiment to experiment.

The second cause is a no less important.

Our numerous observations have shown that phytoncides of one and the same plant vary essentially as to their sterilizing power, according to the part of the plant from which they are obtained. Thus, those produced from the bottom of the bulb are more efficient than those from other parts. It should be taken into consideration, moreover, that onions which were preserved for a long period of time, are less suitable for our purpose. Consequently, experiments that lasted throughout autumn, winter and early spring could not but yield somewhat varying results.

Let us dwell, in conclusion, on a set of experiments which seem to us to be the most illustrative and of some practical interest, too. I have started some tests with the aim to find out the effect of phytoncides upon the bacterial flora of the oral cavity (the experiments were continued by E. Krasnopevzeva). The technique was a quite simple one.

When investigated *in vitro*, some mucus is taken off from the oral cavity with the aid of a sterile sponge or a platinum loop; the mucus collected is seeded in a Petri dish in an agar-agar medium. The experimental dishes are at once treated with the vapour emitted by phytoncides of a certain plant (gruel prepared from the bottom of an onion, garlic, etc.). For this purpose a dish contaning the bacterial culture to be tested was kept bottom upward during 3—10 minutes over another dish, containing a freshly prepared vegetable mass. As a control served dishes with cultures that had not been subjected to phytoncide treatment. Both kinds of dishes were next put into a thermostat at a temperature of 36—38° C. The colonies were kept under daily observation, allowance being made for the dynamics of growth.

Table 2

Bacterial Flora of Mouth Cavity as Affected by Phytoncides

Plant	Number of tests	Exposure, in min.	Number of cases with results			
			Total sterility	Delayed growth	Similar growth of exper. and control	Negative
Allium cepa	20	3	15	1	3	1
» »	20	5	15	5	—	—
» »	10	10	5	3	2	1
Allium sativum	10	3	9	—	1	—
» »	10	5	8	—	1	1
» »	5	1	5	—	—	—
Capsicum	5	3	3	2	—	—
»	5	5	2	—	2	1
»	5	10	1	—	4	—
Pinus pumila	10	1	9	1	—	—
» »	10	3	8	2	—	—
» »	5	5	3	1	1	—
» »	5	10	2	2	1	—
Total	120	—	85	17	15	3

Percentage of positive results—71.

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In another experimental set there was also practised seeding of mucus taken from the oral cavity. The patient was next given to chew some onion or garlic during 1—10 minutes. After this mucus is taken off again and seeded in an identical medium and under identical conditions. It was found that in some cases even one minute of chewing garlic proved sufficient to sterilize the oral cavity completely, while the controls displayed the opulent bacterial growth well known to microbiologists.

The above-said is well illustrated by Table 2, summarizing the results obtained by E. Krasnopevzeva.

Thus, the exceptional bactericidal power of phytoncides leaves no room for doubt anymore.

There still remain, however, many obscure points pertaining to the mechanism of action of phytoncides upon the bacteria and to the possibility of applying to medical practice the data obtained by us and by Tokin's co-workers. Part of these questions, however, lie beyond the author's competence.

Department of Development Dynamics.
Kuibyshev State University at Tomsk.

Received
12. XI.1942.

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[1] А. Филатова и А. Тебянина, Микробиология, 11, 3 (1933).

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OIL OF GARLIC

The bulbs or cloves of the common garlic *Allium sativum* L. (fam. *Liliaceae*) have been used since antiquity, particularly in Mediterranean countries, as a popular prophylactic and curative against all kinds of intestinal ailments (chronic infections of the stomach and the intestines, dysentery, typhoid fever, cholera, etc.), and even against arteriosclerosis and hyperpnea. Phoenician sailors carried good stocks of garlic on their long sea voyages, and the Romans complained about the bad breath of Phoenician emissaries. Because of the considerable curative power of garlic, many attempts have been made to isolate its antibacterial principle. It is only recently, however, that this has been achieved with the identification of *allicin*.

It is a well-known fact that whole (undamaged) bulbs of garlic possess a relatively weak odor. However, when cut up or crushed, the bulbs emit their well-known, powerful and obnoxious odor, due to an essential oil consisting chiefly of disulfides.

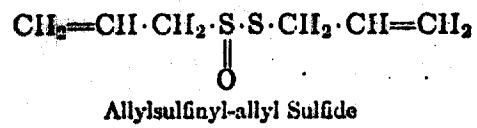
The essential oil of garlic was first prepared more than a century ago by Wertheim,[1] who reported that it consists largely of allyl sulfide $(C_3H_5)S$. However, about fifty years later, Semmler [2] found that the oil contains no allyl sulfide at all, but a number of disulfides and trisulfides, among them chiefly diallyl disulfide (see below). Steam-distilling fresh garlic bulbs, Wertheim, and Semmler, obtained from 0.1 to 0.2 per cent of volatile oil. Distillation of the whole plant gave from 0.05 to 0.09 per cent of oil (Schimmel & Co.).[3]

In 1909 Rundqvist [4] advanced the theory (later disproved) that the parent substance from which the disulfides originate after crushing of the garlic bulbs, is a glucoside. This substance he named *alliin;* he did not, however, succeed in isolating it in pure form.

The actual mechanism of the reaction, by which the antibacterial principle of garlic, and the disulfides in the volatile oil, originate, was finally elucidated only a few years ago through the brilliant work of Cavallito and his collaborators,[5] and Stoll and Seebeck.[6] Their findings can be summarized as follows:

[1] *Liebigs Ann.* **51** (1844), 289; **55** (1845), 297.
[2] *Arch. Pharm.* **230** (1892), 434.
[3] *Ber. Schimmel & Co.*, October (1889), 52; October (1890), 25.
[4] *Pharm. Notisblad* **18** (1909), 323. *Apoth. Ztg.* **25** (1910), 105.
[5] *J. Am. Chem. Soc.* **66** (1944), 1950, 1952; **67** (1945), 1032.
[6] *Experientia* **3** (1947), 114. *Helv. Chim. Acta* **31** (1948), 189; **32** (1949), 197, 866.

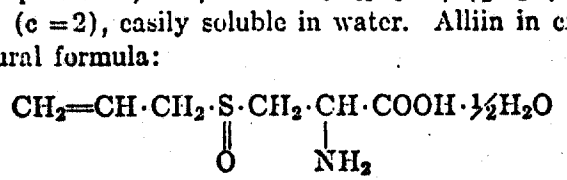
The antibacterial principle of *Allium sativum* L. is *allicin:*



Allylsulfinyl-allyl Sulfide

(For details re Allicin see Vol. II of this work, p. 733.)

Allicin does not occur as such in *whole* garlic bulbs, but in the form of a thermostable precursor, viz., *alliin* $C_6H_{11}O_3NS$, $\frac{1}{2}H_2O$, m. 163°–165°, $[\alpha]_D^{21}$ +62° 4S′ (c = 2), easily soluble in water. Alliin in crystalline form has this structural formula:



Alliin itself does not possess any bactericidal properties. However, when garlic cells are crushed, alliin, under the influence of the lyoenzyme *alliinase,* is rapidly broken down to the antibacterial allicin. The typical odor of garlic appears when the lyoenzyme converts the alliin to allicin.

Allicin is rather unstable; it can be isolated by steam distillation only at reduced pressure. Allicin possesses an odor characteristic of garlic but is not obnoxious. On steam distillation at atmospheric pressure allicin is decomposed to diallyl disulfide and other disulfides, which explains why the volatile garlic oil obtained by steam distillation of the bulbs or of the whole plant consists chiefly of disulfides. In the fermentation cleavage of alliin, the parent substance present in the uncrushed material, allicin is therefore only an intermediary product, being finally converted into the obnoxious disulfides so characteristic of the volatile oil:

$$\text{Alliin} \xrightarrow[\text{Alliinase}]{H_2O} \text{Allicin} \xrightarrow{\text{distillation}} \text{Diallyl disulfide and other sulfides}$$

According to Stoll and Seebeck, allyl sulfenic acid and α-aminoacrylic acid are intermediates in the enzymic splitting of alliin (2 mols.), while pyruvic acid and ammonia, together with diallyl disulfide, are among the end-products.

(For details of the reaction, the reader is referred to the original literature, particularly to the work by Stoll and Seebeck.[7])

Physicochemical Properties of Garlic Oil.—Because of its offensive odor, only a few samples of garlic oil appear to have been examined. Gildemeister and Hoffmann[8] reported these meager data:

[7] *Helv. Chim. Acta* 32 (1949), 197. *Chem. Abstracts* 43 (1949), 3482.
[8] "Die Ätherischen Öle," 3d Ed., Vol. II, 407.

L. is *allicin:*

H=CH_2

p. 733.)

bulbs, but in the form of
NS, ½H_2O, m. 163°–165°,
Alliin in crystalline form

OH·½H_2O

properties. However, when
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g of alliin (2 mols.), while
lyl disulfide, are among the

red to the original literature,
)
Because of its offensive odor,
been examined. Gildemeister

43 (1919), 3482.

Specific Gravity at 15°..... 1.046 to 1.057
Optical Rotation..... ±0°

Shipments of genuine oil of garlic examined by Fritzsche Brothers, Inc., New York, had the following properties:

Specific Gravity at 15°/15°..... 1.055 to 1.008
Refractive Index at 20°..... 1.5578 to 1.5745

Chemical Composition.—Semmler [9] and other workers reported the presence of these compounds in volatile garlic oil:

Diethyl Disulfide. Very small quantities.

Allylpropyl Disulfide(?). The oil investigated by Semmler contained about 6 per cent of a disulfide $C_6H_{12}S_2$, b_{16} 66°–69°, which was probably allylpropyl disulfide (cf. Vol. II of this work, p. 731).

Diallyl Disulfide(?). The chief constituent (about 60 per cent) of the oil was a disulfide $C_6H_{10}S_2$, b_{16} 79°–81°, $d_{14.8}$ 1.0237, probably diallyl disulfide (cf. Vol. II, p. 731).

Diallyl Trisulfide(?). A compound $C_6H_{10}S_3$, b_{16} 112°–122°, d_{15} 1.0845, amounted to about 20 per cent of the oil and was probably diallyl trisulfide (cf. Vol. II, p. 731).

Diallyl Polysulfide(?). The distillation residue contained polysulfides, probably diallyl polysulfide $C_6H_{10}S_4$(?).

According to U. S. Patent 2,554,088 (C. J. Cavallito, 1951), the residue of garlic, obtained by alcoholic extraction, and distillation, contains a bacteriostatic and bactericidal substance identified as *allyl disulfide oxide.*

Use.—Oil of garlic has lately come to be appreciated as a valuable flavoring agent, for use in all kinds of meat preparations, soups, canned goods, and table sauces.

Suggested Additional Literature

A. Stoll and E. Seebeck, "Specific Constituents of Garlic," *Scientia pharm.* **18** (1950), 61.

M. B. Jacobs, "A Study of the Garlic Flavor," *Am. Perfumer* **57** (1951), 301.

[9] *Arch. Pharm.* **230** (1892), 434. Cf. Stoll and Seebeck, *Helv. Chim. Acta* **31** (1948), 189. Cf. Fenerolli, *Rivista ital. essenze profumi* **5** (1923), 28; Laland and Havrehold, *Z. phys. Chem.* **221** (1933), 180.

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PHYSIOLOGICAL EFFECTS OF GARLIC AND DERIVED SUBSTANCES[1]

By

MAXWELL CARL, ROBERT S. McKNIGHT, BERNARD SCOTT, AND CARL C. LINDEGREN

(Received for publication July 25th, 1938)

Vollrath, Walton, and Lindegren (1937) have shown that acrolein is a powerful germicide both in aqueous solutions and as a vapor. Ingersoll, Scott, Vollrath, and Lindegren (1938) have shown that crotonaldehyde is also a powerful germicide and not so readily inhibited by proteins as acrolein. These substances have been tentatively identified as the bactericides in onions and garlic, respectively. Minchin (1927) discussed the therapeutic use of garlic in tuberculosis and obtained positive results clinically. Crossman (1924) discussed the use of *tinctura allii* in the treatment of lobar pneumonia and also reported positive results clinically. The following work was undertaken to determine the physiological effects on experimental animals of the aldehydes, acrolein, and crotonaldehyde and the allyl polysulphides.

The effect of the aldehydes, acrolein, and crotonaldehyde

Different amounts and dilutions of the aldehydes were administered by intraperitoneal injection. Peritoneal injury proved too great, so this method was later abandoned and the aldehydes were administered by stomach-tube. The results with guinea pigs are as follows:

Two animals receiving a 0.5 per cent solution of crotonaldehyde intraperitoneally administered in a dosage of 0.02 gram per kilo of body weight daily, died on the third day.

On reducing the concentration to 0.25 per cent, two animals survived three daily injections when the dose in each injection was raised to 0.03 gram per kilo of body weight.

Another series of determinations were made in which aqueous crotonaldehyde solutions ranging from a 3 per cent down to a 0.3 per cent solution were administered by stomach-tube to white rats. It was found that a 3 per cent solution of crotonaldehyde administered by stomach-tube in amounts corresponding to 0.15 gram per kilo of body weight killed two rats out of three within 6 hours. But when the concentration was reduced to 0.3 per cent, five rats out of seven tolerated doses corresponding to 0.015 gram per kilo of body weight daily for 13 days.

Acrolein is much more poisonous than crotonaldehyde. For example, a 0.5 per cent solution killed on the second day when given intraperitoneally in daily doses of 0.0025 gram per kilo.

A single dose of 0.1 per cent solution of acrolein administered by stomach-tube to two animals in a dose of 0.01 gram per kilo killed them on the first day. However, six animals survived an injection of acrolein by the stomach-tube using a 0.1 per cent solution in doses of 0.005 gram per kilo for at least 9 days.

Lewin (1900) reported the lethal dose of acrolein to be 0.25 gram per kilo, administered as pure aldehyde intrader-

[1] From the University of Southern California, Los Angeles, Calif.

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is urged to investigate its copyright status.

mally. Probably this large dose was walled off by the precipitation of proteins and prevented free entrance into the circulation. Evaporation of the highly volatile acrolein directly from the intradermal pocket may also have reduced the effectiveness of this dose.

The effect of garlic

Five adult *rats* were placed on a diet of alfalfa-meal nutrient mixture to which was added 30 per cent of freshly ground garlic. On the third day, two rats died and the concentration of garlic in the diet was reduced to 20 per cent. On the fourth day, one died and the two survivors were placed on alfalfa meal-nutrient mixture without garlic. On the same day, one died and the lone survivor was placed on 20 per cent garlic diet, dying on the eleventh day.

Ten female *guinea pigs* were placed on diets consisting of alfalfa-meal nutrient mixture to which from 5 to 20 per cent of raw, freshly crushed garlic had been added. Each animal obtained between 20 and 40 grams of the nutrient mixture daily, made up fresh each day. The small quantity was not sufficient to maintain the body weight in most of the animals. This may have caused a predisposition to the poisonous effect of the garlic. However, the diet is ample to sustain life as was shown by a number of control animals. One of the test animals died the second day; two on the eighth; one on the tenth; one on the eleventh; one on the nineteenth; and four were surviving on the twenty-eighth day.

Raw garlic was fed to *mice* to test its effect in cases of pneumococcal peritonitis. Twelve mice were placed for 6 days on diets to which were added, daily, 5 grams of a mixture of cornmeal, powdered milk, and freshly ground garlic. On the seventh day all the mice, except three receiving 10 per cent garlic, were inoculated intraperitoneally with pneumonia sputum. One of three uninoculated animals receiving 10 per cent garlic died on the eighth day. The other nine mice which received 5 per cent garlic died about 40 hours after the pneumococcus injection. Four control mice receiving no garlic died about the same time.

A similar, subsequent experiment involving twenty mice showed no evidence of protection due to the feeding of garlic.

The effects of allyl polysulphides

The allyl polysulphides which are reported to give garlic its characteristic odor were tested to determine the lethal dose. Allyl disulphide containing a small amount of the monosulphide was prepared synthetically by Dr. R. E. Vollrath. Also, a mixture of the higher polysulphides consisting of the tri-, tetra-, and pentasulphides, with the pentasulphide predominating, was prepared. No attempt was made to separate the higher polysulphides because of their instability to heat.

In one experiment eight rats were treated daily by stomach-tube with an emulsion consisting of 2 per cent of polysulphide suspended in a 1 to 1000 aqueous solution of castile soap. Animals receiving 8 cc died about the fifth day; those receiving 2 cc died about the twelfth day.

In another experiment nine rats were each given 1 to 8 cc of the pure polysulphide in a single dose, and nine rats were given the disulphide in a single dose ranging from 1 to 8 cc. All animals given the disulphide died within 10 to 26 hours and all animals given the polysulphide died within 10 to 12 hours.

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In a third experiment two groups of ten animals each were treated with the pure, unemulsified sulphides, but the dosage was reduced to an amount ranging from 0.05 to 0.8 cc. All animals died within 5 days after treatment with the exception of four animals; two receiving 0.1 cc and two receiving 0.05 cc of the disulphide. The lethal dose is about 1.2 grams per kilo.

The amount of allyl polysulphide received by animals fed a garlic diet is comparable with the lethal dose as determined above. As previous investigators have shown, garlic contains about 0.5 per cent of allyl sulphides and this figure was used in comparing the lethal dose of allyl sulphides with the amount of the substance ingested by garlic-fed animals. Garlic also contains crotonaldehyde. The death of the animals on a garlic diet is probably due to the presence of the allyl sulphides rather than the crotonaldehyde in the garlic, but this is difficult to state with certainty.

Unpublished work in this laboratory shows that the dilute emulsions of allyl polysulphides are practically non-bactericidal. Several tests on the blood of animals fed garlic or sulphides did not reveal any bactericidal activity of the blood due to the diet. Morel, Rochaix, Perrot, and Sanlaville (1937) have shown that the sulphides are capable of inhibiting many bacteria.

Preliminary chemotherapeutic observations on acrolein

Artificially infected animals were treated with acrolein to test its chemotherapeutic efficacy. Twenty white rats were inoculated intraperitoneally with pneumonia sputum. Ten were supplied with drinking water containing one part per thousand of acrolein. Two of these rats survived while all ten of the controls died. This encouraging result led to further experimental work. In these tests heart blood of a white rat dead from a pneumococcal infection was used as the inoculum, intraperitoneal injections consisting of 0.05 cc to 0.2 cc of a dilution of the blood in sterile, physiological salt solution being used.

In the first experiment of this series five white rats and five control animals were used.

Daily treatment consisted of the administration by stomach-tube of 1 cc of a 1 to 1000 dilution of acrolein. The heart blood was diluted 1 to 10 and the amount of the injection varied from 0.1 cc to 0.2 cc of the dilution. All controls died and one treated survived.

In a third experiment there were four treated and 4 control animals. Treatment was administered to one group in the form of 0.9 cc of a 1 to 1000 dilution of acrolein in water by stomach-tube. This treatment continued for 7 days when all animals were inoculated with 0.1 cc of a 1 to 100 dilution of pneumococcal heart blood in physiological salt solution. Treatment was discontinued the next day and continued the following day when two treatments were given. One of the treated rats died on the third day after injection. Treatment was continued the next day when all the controls died. Treatment was then discontinued. The remaining three treated rats survived.

In a fourth experiment thirteen treated and thirteen control animals were used. The treated rats received 1 cc of a 1 to 1000 aqueous dilution of acrolein by stomach-tube daily. Treatment was continued for 7 days and all animals were inoculated on the seventh day with 0.05 cc of pneumococcal heart blood in a 1 to 100 dilution. Two of the treated group survived.

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Thus, in these experiments, 32 rats infected with pneumococci and treated with acrolein by various methods, eight survived; while an equal number of similarly infected but untreated rats died.

Summary

The use of a chemical as a therapeutic agent is prevented if the bactericidal dose is more lethal to the host than to the parasite. Since the bactericidal value of garlic, onions, and derived substances had been determined in vitro it was decided to test their toxicity to animals. The sulphides were found to be cumulative poisons to animals, but were only slightly poisonous. The higher aldehydes were not cumulative poisons and were bactericidal in low concentrations. Fresh garlic is very poisonous, probably because of the sulphides and, for this reason, may not be very effective therapeutically. However, there are some indications that the derived aldehydes, especially acrolein, have possibilities as therapeutic agents.

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A Blood Anticoagulant Substance from Garlic(*Allium Sativum*)II.

Chemical Analysis and Studies on the Biochemical* and Pharmacological Effects

Chung Suk Song, Yoon Soo Kim, Dok Je Lee, and Chyong Chik Nam

Department of Biochemistry
Yonsei University College of Medicine, Seoul, Korea

(Received for publication: December 5, 1963)

ABSTRACT

G. E. as prepared in our laboratory is a non-volatile white substance, which is odorless and water soluble. Only in vivo does it have a hypotensive effect, while both in vivo and in vitro it has a hypo-calcemic effect. We determined the chemical analysis, toxicity, lethal dose, and the effect on isolated intestinal and auricular movements of rabbits of G. E. The sodium salt of G. E. contains 18.7% Phosphorus and 15.7% Sodium. It contains inositol and a small amount of sulfur and nitrogen.

The ratio of inositol: phosphorus: sodium is 1:6:6.7. Also G. E. may contain phytic acid and other mat erials which have not been identified. Toxicity tests of G. E. done on mice. The first symptoms of toxicity in mice began with irritability and unstable walking, which were followed by dyspnea and sluggish movements, and finally by coma. Mice LD 50 was 222 mg/kg

As the dose of G. E. was increased in successive injections in the rabbits, the rabbits died, when the total dose reached 100-200mg%. Probably G. E. is not destroyed quickly nor excreted rapidly. The blood pressure in the rabbits continued to fall at each injection indicating no development of tachyphylaxis.

If 70 mg. of G. E. was injected intravenously, as one dose, the rabbit died with muscular hyperactivity. On post mortem examination, we found G. E. had a hypocalcemic effect. However if the calcium salt of G.E. was injected no muscular hyperactivity developed, but severe hypotension was observed. The hypocalcemic effect of G. E. is due to the combining of G. E. with the blood calcium and the muscular activity may be secondary to hypocalcemia.

The G. E. hypotensive effect in atropinized rabbits and in ganglionic blocked rabbits (Hexamethonium) was the same as the effect found in rabbits which had not been drugged. Epinephrine also did not change the hypotensive effect of G. E., G. E. itself showed no effect on the isolated intestinal and auricular movements of a rabbit as long as there were enough calcium ions in the solution. Hence we can not say that the hypotension of G. E. is due to vagus stimulation and or to paralysis of sympathetic nerve endings. The mechanism of the hypotensive effect of G.E. is not yet clear.

INTRODUCTION

Song et al (1963) isolated a blood anticoagulant substance from garlic (This substance is called Garlic Extract or G. E.). G. E. has not only an anticoagulant effect but also is toxic in rabbits. If one hundred milligrams of G. E. are injected into rabbits intravenously, they die.

In order to find the cause of G. E. toxicity, the lethal dose in mice, the change of serum calcium content, and the changes in blood pressure after administration of G. E. were investigated. The effect

* This investigation was supported by a research grant from the Research Council of Yonsei University Medical College.

of G. E. on the heart and intestine were also investigated and a chemical analysis of G. E. was made.

MATERIALS AND METHODS

Sodium salt of G. E.: After the G. E. is dissolved in distilled water and neutralized with sodium hydroxide, alcohol is added, and the resultant is then refrigerated for 30 minutes. The precipitated sodium salt of G. E. is collected and dried.

Calcium salt of G. E.: The sodium salt of G. E. is dissolved in distilled water. Calcium chloride is added in sufficient amounts to precipitate all the G. E. The precipitated calcium salt of G. E. is collected and washed 3-4 times with distilled water and dried.

Hydrolysis of G. E.: 5.0 gm of calcium salt of G. E. was hydrolysed in 50 ml of 6 N hydrochloric acid under a reflux condensor for twenty hours. The hydrochloric acid was removed by the repeated adding and evaporating of alcohol. The residue was dissolved in ten ml. of water. then 5.0 ml of 10% calcium hydroxide solution were added, and the pH adjusted to neutral by adding sodium hydroxide and the mixture was then centrifuged.

The supernatant liquid was collected, alcohol added, and kept in a refrigerator, and if no crystals formed, it was centrifuged to precipitate the phosphate salt. The supernatant liquid conccentrated under reduced pressure. Alcohol was added and this was also kept in a refrigerator. The crystals were collected and recrystalized in a water alcohol mixture.

Hydrolysis of G. E. for quantitative analysis: One hundred mg of the sodium salt of G. E. was dissolved in one ml of 6N hydrochloric acid in a small tube and the tube was sealed and boiled at 110°C for 18~48 hours. The G. E. hydrolysate was transfered to a 100 ml volumetric flask, neutralized with sodium hydroxide and diluted to the mark with distilled water. Two ml of the solution were used for the quantitative analysis for inositol.

The determination of inositol was carried out according to Bühm's method(1954). Paper chromatography for inositol was carried out according to Hubscher's method(1957) except the ascending method in stead of the descending method was used.

The optical activity of G. E. solution was investigated by a Polarimeter.

Animals: White rabbits (body weight 1.5~2.0 kg), mice and frogs were used.

G. E. or Sodium salt of G. E. solution: G. E. or the sodium salt of G. E. is dissolved in distilled water or in 0.9% sodium chloride solution. It is neutralized with sodium hydroxide if necessary.

Calcium salt of G. E. solution: The calcium salt of G. E. is dissolved in distilled water adding hydrochloric acid. After it is completely dissolved, the solution is neutralized. When neutral, the calcium salt of G. E. forms a suspension.

For determination of the lethal dose, male mice weighing from 13 to 18 gms. were used. The G. E. was dissolved in physiological saline, and 0.25~0.5 ml of the solution injected into abdomen.

For investigation of the change in the calcium content of the serum: G. E. solution(2.5~5.0 ml) was injected into the ear vein of rabbits and then the calcium content of the serum was determined.

For investigation of the change in blood pressure: The carotid arterial blood pressure was registered on a smoked drum by means of a mercury manometer. The sodium or calcium salt of G. E. solution (2.0~5.0 ml) was injected into an ear vein in rabbits and the change of blood pressure was chymographed.

Atropine, Hexamethonium and Epinephrine were injected before or after the injection of the G. E. solution and the change of pressure was chymographed.

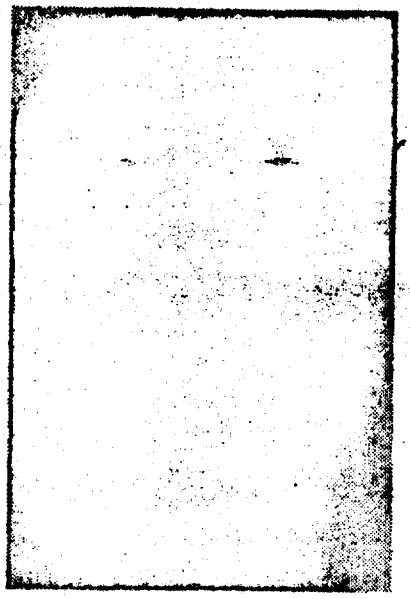
The method of investigation of intestinal and auricular movement of isolated intestine and heart was as follows:

The rabbits were sacrificed and immediately the intenstine was exposured by a midline incision, and about 30 cm of the small intestine was removed. The isolated intestine was soaked in Locke's solution and was cut into sections 2.0~2.5 cm long for chymography by the Magnus method.

Frog's heart movement was studied by the method of Engelman.

In studying auricular movements of the rabbit heart, the fasting rabbit was sacrificed and the heart

was exposuned and removed. The auricle was carefully separated and auricular movement was observed by Langendorff's method.



G.E. Inositol

Fig. 1. Patterns of paper chromatography of the crystal from G. E. hydrolysate and of inositol.

RESULTS

1. Chemical analysis

The Sodium salt of G. E. was found to be optically inactive. The quantitative analysis of phosphorus, nitrogen and sodium gave the following results:

Phosphorus 18.7%, Nitrogen 0.2%, Sodium 15.7%.

In addition to these elements, a small amount of sulfur was detected. The crystals obtained from the sodium salt of G. E. hydrolysate were similar to inositol and were compared with pure inositol. The resust is shown in Table 1 and in Fig. 1.

Table 1. Comparison of the crystals from G. E. hydrolysate with inositol

	Taste	Melting Point (centigrade)	Periodate consumed per 300 r.(r.Mol)	Paperchromatography Rf
Crystals fromG.E. hydrolysate	Sweet	125	9.7	0.28
Inositol, pure	Sweet	125	9.7 (10.0)*	0.28

* Estimated r. Mole for 300 r. of Inositol

Quantitative analysis of inositol in G. E. hydrolysate by Böhm's method indicated that 975 gm of sodium salt of G. E. contained one mole of inositol.

From these studies, the ratio of inositol:phosphorus:sodium of sodium salt of G. E. was found to be 1:6:6.7.

2. Determination of the lethal dose

A) The symptoms of toxicity

One minute after the injection of 2.4 mg of G. E. /10 gm of body weight into abdominal cavity, the mice started to walk lifting the posterior legs alternately.

Two minutes later, the mice walked in a crouched position. Three minutes later, they ceased walking and frequently moved their anterior legs. Five minutes later, they lifted their tails. Seven minutes later, they developed dyspnea and restlessness and walked sluggishly. Four mice out of ten died in this state in 13 minutes, but the others recovered from the acute symptoms. 22 minutes later, similar symptoms were observed again in other group: 2 minutes later, after the second attack, slight restlessness were noticed. 5 minutes later, the mice in a crouched position and respirations were increased. 12 minutes later, they were in an unstable position, showed restlessness and difficulty in walking. 15 minutes later, they were in lying down, and some of these died in this state. The others soon recovered completely.

B) The lethal dose was determined according to the method of Behrens-Kärber. The results are shown in Table 2.

Table 2. Lethal dose

mg of G. E. injected per 10 gm. of body weight	1.9	2.0	2.1	2.2	2.3	2.3	2.5
No. of mice injected	10	10	10	10	10	10	10
No. of mice killed	0	4	4	5	5	4	10

L.D. 50=222(mg/kg)

3. The effect of G. E. on the calcium content of the blood

150 mg. of G. E. was injected into the ear vein of a white rabbit(1.8 kg of body wegiht). The rabbit soon died showing hypermuscular activity. Blood was collected from the heart by a syringe before injection and again after death. The calcium content

Fig. 2. Pattern of the blood pressure and respiration response to each injection of G. E. in rabbit.

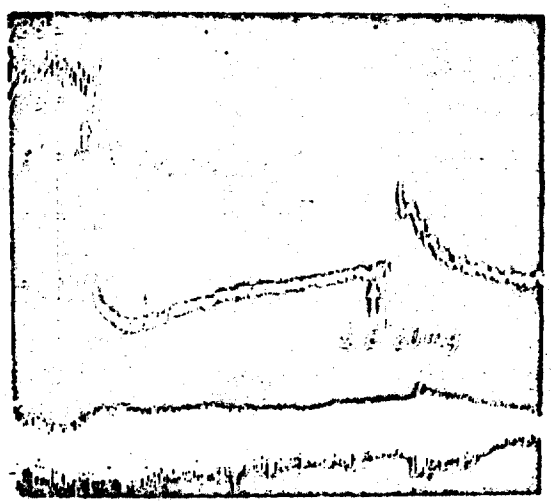


Fig. 4. A modification of the blood pressure response to G. E. due to Hexamethonium (5 mg/kg) and G. E.(20 mg/2kg) were each given.

in the serum before injection was 12.4 mg, after injection the calcium content was reduced to 2.9 mg%.

4. The effect of G. E. on the blood pressure

A) The pattern of the blood pressure response in rabbits.

10 mg, 20 mg and 30 mg of G. E. in saline sucessively were injected intravenously into rabbits, after each injection the blood pressure abruptly fell and soon rose again to the original pressure, or to a level higher than that of the original pressure. see Fig. 2

As the rabbit responded to each injection of G. E., it was not likely to develop tachyphylaxis. How-

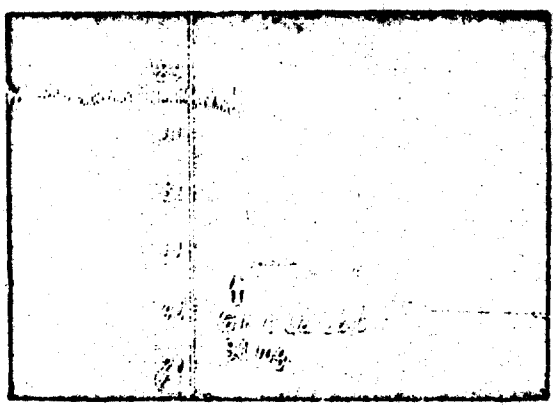


Fig. 3. Pattern of the blood pressure response to the calcium salt of G. E. in rabbit.

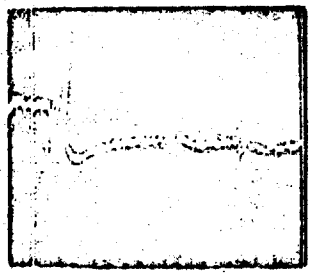


Fig. 5. A modification of blood pressure response to G. E. due to atropine(20 r./kg). 30 minutes after the injection of atropine, 30 mg of G. E /2kg was given.

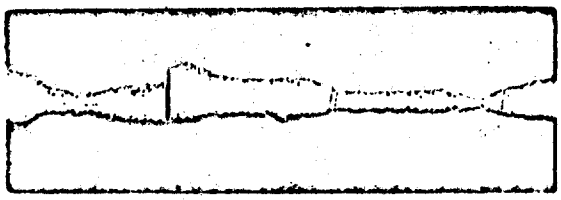


Fig. 6. The effect of G. E. on the auricular movement in the isolated heart of a rabbit.

Fig. 7. The effect of G. E., and calcium salt of G. E. on the intestinal movement in the isolated rabbit intestine.

ever, after successive injections, the response was increased and when the total dose of G. E. reached to 100-200 mg, the rabbits died. It can be said that G. E. has a cumulative effect.

When a moderate dose of G. E. (30 mg) was injected, respiratory disturbances occured. When a large dose(about 70 mg) was injected at one time, the blood pressure fell to zero and the rabbits died.

The calcium salt of G. E. showed greater effect on the blood pressure than did G. E. itself. 15~30 mg. of calcium salt of G. E. killed the rabbits and its hypotensive effect as shown in Fig. 3 was very great.

B) Resonse of rabbits atropinized or treated with hexamethonium or epinephrine.

As is shown in Fig. 4 and 5. G. E. showed the same hypotensive effect in atropinized rabbits as in rabbits treated with hexamethonium. It was the same as in untreated rabbits. These drugs did not change the effect of G. E. on the blood pressure. Epinephrine also did not change the hypotensive effect of G. E.

C) The effect on auricular movement in isolated rabbit heart preparations.

As is shown in Fig. 6, 0.05% of G. E. inhibited the contraction of the auricle, but when calcium chloride was added drop by drop, the contraction returned to normal. The calcium salt of G. E. suspension had no effect on contraction.

D) The effect on the frog heart.

G. E. solution showed no effect on frog's heart movement. Even when a large dose of G. E. was injected into a frog's superficial abdominal vein, there was no effect on heart, nor did it kill the frogs.

E) The effect on intestinal movements of rabbits.

As is shown in Fig. 7, as the concentration of G. E. was elevated, it inhibited intestinal movements but when calcium chloride was added drop by drop, the. intestinal movements returned to normal.

The calcium salt of G. E. in the same concentration, had no effect on intestinal movement.

DISCUSSION

Hans Platenins(1935) isolated a volatile compound containing sulfur from Allium savitum. Another investigator, Abe(1938) precisely analysed the sulfur compound, and separated propylsulfide, diallyl disulfide and diallyl trisulfide.

Dittner(1929) stated that the allyl sulfide of the volatile compound has a antibacterial action and termed it allicin. It affected gram(+), gram(-) bacillae. He also confirmed that 1 mg of allicin was equivalent to 15 oxford units of penicillin. He analysed its chemical structure as $C_3H_5S(=O)SC_3H_5$ which is converted into $(C_3H_5S)_2$ by allicinase or by steam distillation.

Sgihara(1926) who separated a volatile substance from garlic demonstrated that it had a hypotensive effect and he thought that this hypotensive effect was due to depression of the central nervous system. This volatile substance was investigated by Kahwara and its chem icals tructure was found to $C_6H_{12}S_2$. This was found to be allicin by Cavalito, et al. (1944) 37years later, G. E. was separated from garlic in our laboratory. It has a hypotensive effect but is a non-volatile substance and so this material is quite different from the volatile substance separated by Sgihara.

When G. E. was injected intravenously into rabbits, it showed a hypotensive effect. The blood pressure fell abruptly with dyspnea and increasing muscular activity. G. E. has a hypocalcemic effect also, which is due to the powerful combination of G. E. with ionized calcium in blood. The muscular hyperactivity after G. E. injection might be due to a secondary hypocalcemia.

When the calcium salts of G. E. were administered by the same method, respiratory andmuscular hyperactivity were not observed.

In isolated intestine and heart muscle, G. E. inhibited the movement of the intestine and contraction of the heart but when calcium chloride was added, movement and contraction returned to normal. Calcium salts of G. E. showed no effect on heart contraction nor on intestinal movement. From these facts, it may be said that the effect of G. E. on the intestine and the heart is due to precipitation of calcium ions from the solution.

G. E. hypotensive effect in atropinized rabbits, and in hexamethonium ganglionic blocked rabbits is the same as in untreated rabbits. This is also

true with epinephrine. So we can not say that the hypotensive effect of G. E. is due to stimulation of the vagus endings or to paralysis of the sympathetic nerve endings. The mechanism of the hypotensive effect of G. E. is not yet clear.

As the total dose of G. E. on successive injections reached to 100-200 mg the rabbits died. G. E. may not be destroyed quickly nor excreted rapidly and the rabbits did not develop tachyphylaxis.

Concerning chemical analysis, G. E. contains inositol and phosphate, and the ratio is one to six, which means that G. E. may contain phytic acid. As the sodium salt of G. E. is not a pure material there is the possibility, that other substances are combined in it. Further investigation is necessary to find the active agent.

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LETTERS TO THE EDITOR

Of the methyl substituted procaine congeners, 2-diethylaminoethyl-3 5-dimethylbenzoate hydrochloride was most effective having a therapeutic index of 64·1 and a protective index of 28·5.

2-Diethylaminoethyl-2,3-dimethylbenzoate hydrochloride and 2-diethylaminoethyl-2,3,5,6-tetramethylbenzoate hydrochloride had higher protective indices, but lower therapeutic indices compared with procaine hydrochloride.

Compared with procaine hydrochloride, 2-diethylaminoethyl-2,5-dimethylbenzoate hydrochloride has slightly higher therapeutic index (37·4), but a much higher protective index.

The present study reveals that methyl substitution at position 3 and 5 in the benzene ring of procaine yields a compound which has much higher protective and therapeutic indices compared with the parent substance procaine.

Acknowledgement. We thank the Director, A.I.I.M.S. for facilities, the I.C.M.R. for financial support, Messrs. McNeil Laboratories for some of the procaine congeners, Messrs. Parke Davis and Co. for phenytoin sodium, Messrs. Knoll for Metrazol and Mr. T. N. Sugathan for the statistical analysis.

Department of Pharmacology, KANTI KAPILA.
All-India Institute of Medical Sciences, R. B. ARORA.
New Delhi-16, India.
February 13, 1962.

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Orally Effective Hypoglycaemic Agents from Plants

SIR,—Hypoglycaemic agents from *Allium cepa* Linn. (the domestic onion), *Ficus bengalensis* Linn. and *Eugenia jambolana* Lam. have already been reported by us (1961). The present communication describes two more orally effective hypoglycaemic principles extracted from *Allium sativum* Linn. (garlic) and from an Indian indigenous plant, *Ficus religiosa* Linn.

TABLE I

BIOLOGICAL ASSAY OF ORALLY EFFECTIVE HYPOGLYCAEMIC AGENTS FROM *Allium sativum* AND *F. religiosa*. COMPARED WITH TOLBUTAMIDE

Substance administered	Dose	Blood sugar response mg./100 ml.			
		Initial average values for six rabbits	4 hr. pool average values for six rabbits	Mean reduction per cent	Mean hypoglycaemic potency as per cent of tolbutamide
Tolbutamide (Albert David and Co.)	0·5 g.	100	74·98	25 ± 2·1	100
Total ethyl ether (34–36°) extract from 50 g. dry garlic powder ..	0·5 g.	117·3	100·03	14·72 ± 3·5	58·88
Total water extract from 50 g. dry root bark powder of *F. religiosa*..	2·5 g.	117·9	95·52	18.97 ± 4·34	75·9

The hypoglycaemic effect of the different fractions of garlic extracts was reported by Laland and Havrivold (1933). *F. religiosa* Linn. is used throughout India as a natural remedy for diabetes mellitus as mentioned by Chopra (1933).

(32)

Sun dried cloves of
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given in Table I. Ti
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1 hr. before the admin

Blood glucose mg./100 ml.

FIG. 1. Effects of the
fasting rabbits. (X—
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The results, Table I
contain orally effectiv
A detailed report
hypoglycaemic agents

Department of Bioche
Birla College,
Pilani (India.)
January 29, 1962.

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Chopra, R. N. (1933).
Mukherjee & Co.
Folin, O. and Malmros
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LETTERS TO THE EDITOR

Sun dried cloves of garlic and the root bark of *F. religiosa* were thoroughly dried and extracted separately with different solvents in soxhlets. Only the water extract of *F. religiosa* and the ethyl ether extract of garlic were found to exhibit sufficient hypoglycaemic activity, on oral administration to rabbits, compared to a standard dose of tolbutamide (0·5 g./rabbit). The results of the biological assay of these extracts on groups of normal male albino rabbits weighing 2 kg. with fasting 18 hr. blood sugar levels of 100–125 mg./100 ml. are given in Table I. The biological assay was made by a procedure similar to that of Marks (1926). Blood sugar was determined by the micromethod of Folin and Malmros (1929).

Both these extracts were found to be effective in controlling the hyperglycaemic response of glucose feeding (1 g./kg.) in glucose tolerance experiments on normal fasting rabbits, as will be evident from Fig. 1. The drugs concerned were fed 1 hr. before the administration of glucose to facilitate their absorption.

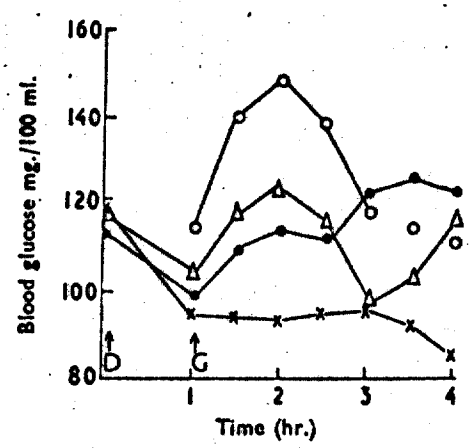

FIG. 1. Effects of the hypoglycaemic agents on the glucose tolerance of normal fasting rabbits. (X——X) tolbutamide; (O——O) *Allium sativum*; (△——△) *Ficus religiosa*; (○——○) control. Dose same as given in the Table I. Each curve is a mean of results from six rabbits. D = drug, G = glucose.

The results, Table I and Fig. 1, show that, *Allium sativum* and *F. religiosa* contain orally effective hypoglycaemic agents.

A detailed report on the separation and isolation of the orally effective hypoglycaemic agents from these natural sources will be published elsewhere.

H. D. BRAHMACHARI.
K. T. AUGUSTI.

Department of Biochemistry,
Birla College,
Pilani (India.)

January 29, 1962.

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Brahmachari, H. D. and Augusti, K. T. (1961). *Ibid.*, 13, 381–382.
Chopra, R. N. (1933). *Indigenous Drugs of India*, 1st Ed. p. 490, Calcutta: N. Mukherjee & Co.
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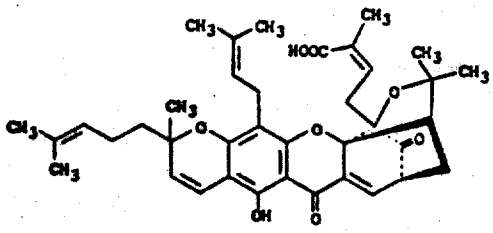
USE: Tanning, dyeing fabrics brown or black.
MED USE: Has been used as astringent.
VET USE: Astringent in diarrhea. *Dose:* horses and cattle: 15–30 g; foals, calves, sheep and swine: 4–8 g; dogs: 0.3–2.0 g.

Gamboge. Cambogia. Gum-resin from *Garcinia hanburyi* Hook. f., *Guttiferae*. *Habit.* East Indies. *Constit.* 70–80% resin, cambogic acid, 15–25% gum.
MED USE: Drastic cathartic.
VET USE: Drastic cathartic for cattle. *Dose:* cattle: 15–30 g.

Gambogic Acid. $C_{38}H_{44}O_8$; mol wt 628.73. C 72.59%, H 7.05%, O 20.36%. Principal acidic component of gamboge from the latex of *Garcinia hanburyi* Hook. f., *Guttiferae:* Amorosa, Lipparini, *Ann. Chim. (Rome)* 45, 977 (1955). Structure: Ollis *et al.*, *Tetrahedron* 21, 1453 (1965); Ahmed *et al.*, *J. Chem. Soc.* (c) 1966, 772.



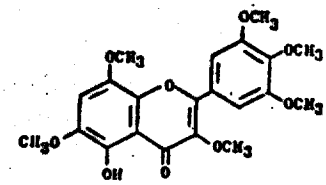
Golden, friable mass. $[\alpha]_D^{20}$ −685° (in methanol). Absorption max in ethanol: 217, 280, 291, 362 mμ (ε 26,000; 16,700; 17,000; 14,900).
Methyl ester monomethyl ether, $C_{40}H_{48}O_8$, yellow prisms from methanol, mp 130–131°. $[\alpha]_D^{22}$ −560° (c = 0.7 in chloroform). Absorption max in ethanol: 224, 299 mμ (ε 36,000; 13,600).
Pyridine salt, $C_{38}H_{44}O_8.C_5H_5N$, orange needles from ether + petr ether, m [illegible] $[\alpha]_D$ −550° (c = 1.0 in chloroform). Absor [illegible] 2915, 3595 Å (ε 22,300; 18,100).

Ganglerone. *p-Is dimetilpropyl este* isobutoxybenzoate; γ-diethylaminopro C 71.60%, H 9.92 oyan *et al.*, U.S.S

they consist of fatty acids (mainly stearic acid), sphingosine, hexoses (galactose and glucose), galactosamine, and sialic acid. Isoln from normal brain and from the brain of patients with Tay-Sachs disease: Klenk, *Z. physiol. Chem.* 265, 50 (1941); 273, 76 (1942); Trams, Lauter, *Biochem. Biophys. Acta* 60, 330 (1962); Svennerholm, *Acta Chem. Scand.* 17, 239 (1963). Occurrence of different gangliosides: Kuhn, Egge, *Angew. Chem.* 72, 805 (1960). Chromatographic studies: Kuhn *et al.*, *ibid.* 73, 580 (1961); Klenk, Gielen, *Z. physiol. Chem.* 326, 144 (1961). Structure of gangliosides G_I: Kuhn, Wiegandt, *Ber.* 96, 866 (1963). Structure of gangliosides G_I and G_{II}: Kuhn, Egge, *ibid.* 96, 3338 (1963). Structure of gangliosides G_{II}, G_{III} and G_{IV}: Kuhn, Wiegandt, *Z. Naturforsch.* 18b, 541 (1963).
Ganglioside G_I: R = Lactaminic acid *see* under sialic acid, R′ = H. $C_{73}H_{121}N_3O_{31}$.
Ganglioside G_{II}: R = R′ = Lactaminic acid. $C_{86}H_{146}N_4O_{38}$.

Ganomalite. 2CaO.3PbO.3SiO₂—calcium lead silicate.

Gardenin. *5-Hydroxy-3,3′,4′,5′,6,8-hexamethoxyflavone;* 5-hydroxy-3,6,8,3′,4′,5′-hexamethoxyflavone; 3,3′,4′,5′,6,8-hexamethoxy-5-hydroxyflavone. $C_{21}H_{22}O_8$; mol wt 418.39. C 60.28%, H 5.30%, O 34.42%. From gum of *Gardenia lucida* Roxb., *Rubiaceae:* Stenhouse, Groves, *J. Chem. Soc.* 1877, 552; *Ann.* 200, 311 (1880); Balakrishna, Seshadri, *Proc. Indian Acad. Sci. Sect. A* 27, 91 (1948). Structure: Bose, *J. Indian Chem. Soc.* 22, 233 (1945). Synthesis: Ahluwalia *et al.*, *J. Chem. Soc.* 1954, 3988.



Golden-yellow needles from dil alc, mp 163–165°. Soluble in alcohol, chloroform.
[illegible]-dehydrogardenin, $C_{21}H_{24}O_8$, crystals, mp 116–117°.

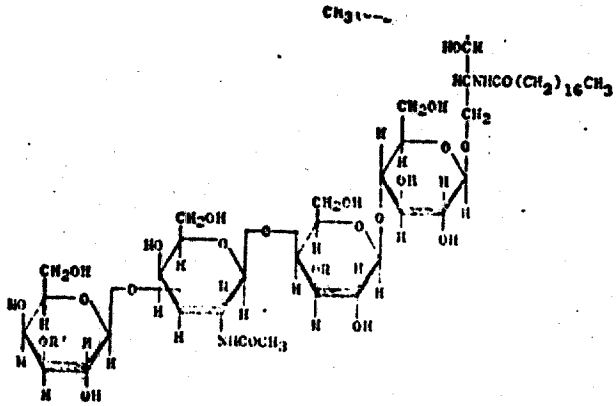
[illegible]ents. A mixture of the sodium [illegible] by reducing the mixed [illegible] and of fish oils, [illegible], wool fat, and [illegible] of the sulfated "lauryl alcohol" [illegible] octyl and decyl) [illegible] cohol, 30% C_{14} [illegible], mixed C_{16} and [illegible]

[illegible] type of detergent [illegible]*l; Maprofix; Ter-Sapidan; Lissapol;* [illegible] dispersing agents fungicidal sprays, in [illegible] textile manuf. *Human* [illegible] tivity reactions.

Merck Index 8th Ed. 1968

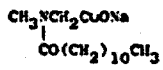
Used as th
MED USE: [illegible]

Gangliosi
grey and white matter [illegible]



ine Na-salt; sodium [illegible]lan LL-99. $C_{15}H_{28}$- C 61.41%, H 9.62%, [illegible]%. Prepn: Jungermann [illegible] 172 (1956).

[illegible] *et al.*, *J. Am.*



USE: As detergent, foaming agent and antienzyme for dentifrices: King, U.S. pat. 2,689,179 (1954 to Colgate-Palmolive).
Note: An aq soln is marketed under the trade name [illegible]

Garlic. Allium. Fresh bulb of *Allium sativum* L., *Liliaceae*. *Habit.* Central Asia, Southern Europe, U.S. *Constit.* Alliin, allicin, volatile and fatty oils, mucilage, albumin.
USE: As a spice and seasoning in foods.

Water. Determine by the *Karl Fischer Titrimetric Method*, page 804.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Acidifier; flavoring agent.

GARLIC OIL

DESCRIPTION

The volatile oil obtained by steam distillation from the crushed bulbs or cloves of the common garlic plant, *Allium sativum* L. (Fam. *Liliaceae*). It is a clear reddish orange liquid, having a strong pungent odor, and flavor characteristic of garlic. It is soluble in most fixed oils and in mineral oil. It may be incompletely soluble in alcohol. It is insoluble n glycerin and in propylene glycol.

SPECIFICATIONS

Refractive index. Between 1.5590 and 1.5790 at 20°.

Specific gravity. Between 1.040 and 1.090.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 per cent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 per cent).

Lead. Not more than 10 parts per million (0.001 per cent).

TESTS

Refractive index, page 785. Determine with an Abbé or other refractometer of equal or greater accuracy.

Specific gravity. Determine by any reliable method (see page 4).

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 720.

Heavy metals. Prepare and test a 500-mg. sample as directed in *Method II* under the *Heavy Metals Test*, page 763, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 772, using 10 mcg. of lead ion (Pb) in the control.

Packaging and storage. Store in full, tight, preferably glass, or aluminum containers in a cool place protected from light.

Functional use in foods. Flavoring agent.

SCIENTIFIC SECTION
ESSENTIAL OIL ASSOCIATION OF U. S. A.
2 Lexington Ave., New York 10, N. Y.

E.O.A. STANDARD for: OIL GARLIC

Oil Garlic is the volatile oil prepared from the crushed bulbs or cloves of the common garlic Allium sativum L. (fam. Lilaceae).

Oil Garlic has a powerful odor and flavor and is used primarily in flavors.

Since Oil Garlic is purchased mainly for its odo and flavor quality, any physical constants are neces sarily limited in value. The most important examina tion is strictly organoleptic.

SPECIFICATIONS

Botanical Nomenclature	*Allium sativum L.* (Family: *Liliaceae)*
Preparation	Obtained by the steam distillation of the crushed bulbs or cloves.
Physical and Chemical Constants	Appearance and Odor: Clear reddish orange liquid with a strong pungent characteristic odor of garlic. Specific Gravity at 25°/25°C: 1.040 to 1.090. (Temperature correction factor from n°/n°C: 0.00058 per °C.) Refractive Index at 20°C: 1.5590 to 1.5790.
Descriptive Characteristics	Solubility: Alcohol: Fairly soluble in alcohol. Benzyl Benzoate: Soluble in all proportions. Diethyl Phthalate: Soluble in all proportions. Fixed Oils: Soluble in all proportions in most fixed oils. Glycerine: Not soluble. Mineral Oil: Soluble in all proportions. Propylene Glycol: Not soluble. Stability: Acid: Relatively stable in the presence of weak organic acids. Alkali: Relatively stable in the presence of weak alkalies.
Containers	Ship preferably in glass. Aluminum containers may be used.
Storage	Store preferably in tight, full containers in a cool place, protected from light.

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of worms in 3, 5 and 10 minutes respectively. From the above, it is clear that the petroleum ether extract possesses anthelmintic activity although it is not quite marked. Psoralen and psoralen-isopsoralen mixture showed fairly good activity. No synergistic activity was found in the mixture of psoralen-isopsoralen. The antibacterial principle showed less activity than psoralen. Comparison of psoralen with hexylresorcinol revealed that psoralen is not a powerful anthelmintic.

The authors wish to thank Dr. M. L. Dhar, Director, Central Drugs Research Institute, Lucknow, for the supply of pure samples of psoralen and psoralen-isopsoralen mixture.

Department of Pharmacy,
Panjab University,
Chandigarh-3.
28 December, 1964.

K. N. Gaind
R. N. Dar
B. M. Chopra
R. N. Kaul

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5. Gaind, K. N., Dar, R. N. and Kaul, R. N., *Indian J. Pharm.*, 1964, 26, 141.

SPECTROPHOTOMETRIC ESTIMATION OF ALLIIN IN ALLIUM SPECIES

A colorimetric method for the estimation of alliin in allium is described. The method is based on the formation of a pink-violet colour complex of alliin with alloxan. The colour intensity of alliin-alloxan complex is measured at 520 mμ.

The genus *Allium* (garlics) includes a large number of species, commonly used as an article of food and condiment. Among the ancients, garlic was credited with curative virtues in an extraordinarily large variety of diseases and its preparations have been used in medicine in a wide variety of ailments.[1,2] An unstable compound "allicin" was isolated from *A. sativum* possessing marked antibacterial activity. In recent years, a crystalline principle alliin, a precursor of allicin, has been isolated[3,4] from *A. sativum* and *A. ursinum*.

Occurrence of alliin in Indian allium was reported by Rao *et al.*[5] and Atal and Sethi.[6] The latter also developed a method for the semi-quantitave determination of alliin in alliums. The present note describes a procedure for quantitatively estimating the amount of alliin present in Indian alliums.

Allium sativum (white) was procured from the local market, *A. sativum* (red) from Nahan (H.P.) and a triploid *Allium* species[7] (Pran) from Kashmir.

General procedure: To each of a set of solutions containing 40 μg., 80 μg., 120 μg., 160 μg. and 200 μg. of pure alliin in methanol was added 2 ml. of 0.25% alloxan[8] (recrystallized) solution in methanol. The final volume in each case was adjusted to 10 ml. with suitable quantities of methanol. The solutions were then heated on boiling water-bath till all the methanol was evaporated, leaving behind a viscous residue. After cooling, the volume was adjusted to 10 ml. with distilled water. The absorbance was then measured in a Hilger Uvispek at 520 mμ against a blank.

A calibration curve prepared from a set of such readings indicated that Beer's law was obeyed over a concentration range of 5-20 μg./ml.

Estimation in allium (white): Atal and Sethi have reported that alliin could be detected only when whole bulbs are stabilized by immersion in boiling water for 5 minutes prior to extraction, in order to destroy the enzyme allinase.[9]

Stabilized bulb (10 g.) was homogenized in a Waring blender with 40 ml. of 70% ethyl alcohol. The resulting mixture was filtered and the volume of the filtrate was adjusted to 40 ml. by the addition of 70% ethanol. With a micro-pipette, 0.04 ml. of this extract was applied on a

FOR PURPOSES OF STUDY OR RESEARCH IN LIEU OF LENDING THE ORIG

sheet of Whatman no. 1 chromatographic paper. On the starting line of the same sheet of paper one spot of pure alliin solution in 70% ethanol was applied. After the development of the chromatogram for 24 hours at room temperature (26°) using butanol-acetic acid-water (40:10:50) system, the spot given by pure alliin (Rf., 0.29) was produced with 0.25% ninhydrin reagent solution, suitably covering the other half of the paper where the crude extract was applied. A strip of the paper in front of the pure alliin spot was cut and alliin was eluted with methanol. The volume of the eluate was adjusted to 10 ml. with methanol and the alliin was determined as in the general procedure.

1.7 mg. of pure alliin was dissolved in 5 ml. of 70% ethanol. 0.5 ml. of this solution was applied to a small strip of chromatographic paper and the strip after drying, was properly hung at the end of a condenser tube fitted to a round-bottom flask containing methanol. Refluxing for 5-6 hours was found to be sufficient to elute out alliin from the strip. The amount of alliin was then determined as in the general procedure. The amount of alliin recoved was 1.68 mg., which is 99% of the original amount used.

A similar procedure was adopted in the case of *A. sativum* (red) and *Allium*-species (Pran).

From the results thus obtained, *A. sativum* (local white), *A. sativum* (H.P. red), and *Allium* species (Pran), were found to contain 0.76%, 0.57% and 0.07% of alliin respectively.

Thus it has been shown that the white variety of *A. sativum* contains the maximum amount of alliin as compared to other varieties.

The earlier method reported by Atal and Sethi is of semi-quantitative nature. However, present method is accurate and sensitive and it is possible to find out even as little as 4 μg./ml. of alliin in the extract.

Our thanks are due to the Director of Research, Sandoz Ltd., Basle, Switzerland, for the supply of pure alliin sample and Mr. J. L. Khoshoo, Retired Conservator of Forests for the supply of Pran variety of allium from Kashmir.

Department of Pharmacy,
Panjab University,
Chandigarh-3.
28 December, 1964.

K. N. Gaind
R. N. Dar
S. D. Popli

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ESTIMATION OF PHENOL IN VACCINES

The titrimetric method for the determination of pure phenol is described in the pharmacopoeias. However, no procedure is outlined for the estimation of phenol added as preservative in biological materials. This communication describes the adaptation of the phenol assay method decribed in British Pharmacopoeia (1963)[1] for the estimation of added phenol in rabies vaccine.

The 0.1 *N* bromine solution which has to be standardized frequently can be conveniently replaced by the brominating mixture[2] consisting of 75 g. of potassium bromide and 5.6 g. of potassium bromate (0.2 *N*). Rabies vaccine preparations were referred by the Virus Department, Haffkine Institute.

Procedure: The extracts are prepared by mixing 1 ml. aliquots of vaccine with: (i) 9 ml. of normal saline, (ii) 9 ml. of 10% trichloroacetic acid (TCA), and (iii) 4.5 ml. of 10% sodium tungstate + 4.5 ml. of 0.67 *N* sulphuric acid.

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SPICES VOL. I

1920

FENUGREEK SEED

Fenugreek seeds are the product of *Trigonella foenum-graecum* L., an annual herbaceous plant belonging to the *Leguminosae*, or Pea family, and native to southern Europe.

Fenugreek is cultivated in France, Lebanon, Morocco, India, and Argentine.

The plant reaches a height of about two feet, and has light-green leaves and small, white flowers. The fruit consists of long, narrow, curved pods terminating in long, slender points. The seeds are yellowish-brown, flat, almost square-shaped, each marked with a deep furrow. They have an aroma reminiscent of burnt sugar, and a mealy taste.

Fenugreek seeds are available whole or ground. They are used in the preparation of imitation maple extract, pungent mango chutney and green mango chutney. The ground seed is an ingredient of curry powder.

GARLIC

Garlic is the compound bulb of *Allium sativum* L., a perennial plant belonging to the *Liliaceae*, or Lily family. It is indigenous to Europe and enjoys almost universal cultivation.

The compound bulb consists of a number of wedge-shaped bulblets, often called "cloves." The leaves of the garlic plant are long, narrow, flat, and sheath the lower part of the stem. The flowers are purple in color and produced in a cluster at the termination of the stem.

The spice consists of (1) garlic powder, which is the ground product of dehydrated cloves of garlic, and is a creamy-white powder with a strong, persistant aroma and taste; and (2) garlic salt, which is the ground dehydrated garlic mixed with free-running table salt and sometimes with a small quantity of edible starch to prevent caking. In addition to the above, minced dehydrated garlic is now available.

Garlic spice products have innumerable culinary uses including the flavoring of soups, meats, gravies, sauces, salad dressings, and garlic bread. Garlic salt goes well with tomato juice, and on steaks, stews and other meat dishes. Garlic powder is employed commercially in the processing of fancy meats and sausages.

Garlic spice products readily absorb moisture and their containers should be tightly capped when not in use.

Experiments on Using Phytoncides with a Medicinal and Prophylactic (Preventive Treatment) Purpose

Candidate in Medical Sciences, Lieutenant Colonel, Medical Section, M.N. Fortuna From the Suvorovsk Military Academy (Director of the Medical Section--Colonel, Medical Section, N. N. Polushkin)

Among the outstanding achievements of Soviet medicine should be included the discovery of B. P. Tokin, who, in 1928, disclosed in plants substances that he called phytoncides; this pointed out their plant origin. It has been established that phytoncides bring about the destruction of the pathogenic organisms of many diseases. The discovery of Prof. Tokin represented a great contribution to the problem of public health care. It was instrumental in the appearance of a new direction in medicine -- the use of phytoncides for the medical treatment and prophylaxis (preventive treatment) of various diseases.

Our observations, which had been carried out over a period of 2 years, confirmed the data of Tokin with regard to the fact that the phytoncides of many higher plants, including fruits and vegetables, bring about the destruction of the simplest organisms (infusoria, amoeba, and others). There was also confirmed the bactericidal action of phytoncides on a culture of hemolytic streptococcus, dysenteric bacillus of the Flexner type, grippe virus of the A and B types, and salmonella. According to our data, the phytoncides of onion, garlic and horseradish have manifested a bactericidal effect on a culture of hemolytic streptococcus; the phytoncides of garlic -- on a dysenteric bacillus of the Flexner type the phytoncides of ionin, dog (hip-bearing) rose and garlic -- on a grippe virus of the A and B types; the phytoncides of onion and garlic -- on salmonella. It has also been established, by our observations, that there is a stimulating action on the part of the phytoncides of garlic on Bacillus coli, which, as is known, represents an antagonist of pathogenic flora. By studying the action of phytoncides on an isolated frog heart, we established that phytoncides possess a high degree of activity. Thus, the phytoncides of garlic manifested their action in dilutions of $10^{\text{minus } 18}$, the phytoncides of onion -- in dilutions of $10^{\text{minus } 25}$,

and the phytoncides of horseradish -- in dilutions of $10^{\text{minus } 75}$. On the basis of the data in the literature, and on that of our own observations, we started to make use of phytoncides with a prophylactic (preventive treatment) and medici nal purpose in a number of illnesses in adults and in children. Thus, for the medical treatment of angina, we used the phytoncides of onion and garlic by means of spraying the pharynx (throat). For this purpose, ionion juice was dilu ed with a physilogical solution, 5 times, and that of garlic -- 10 times. The spraying was carried out 3 times per day. In comparing the action of streptocid and that of phytoncides, we noted that the latter manifested a better action according to a number of characteristics: there was a more rapid disappearance of the occurrence of pharyngeal (throat) hyperemia and of pains on swallowing; ther was a more rapid drop in the temperature; and there was a reduction in the numbe of days in bed. The same result was also obtained in the case of the effect of greater dilutions of garlic phytoncides ($10^{\text{minus } 18}$).

For the prophylaxis (preventive treatment) of angina we made use of phytoncides of onion and garlic, comparing their action with the action of penicilli The duration of medication was equal to 3 days. As a criterion, there served th frequency of cases of illness with angina over a period of a month following the termination of medication. It has been established that in a group in which the medication was not carried out, thefrequency of illnesses with angina comprised 8.7%, and in a group medicated with phytoncides and penicillin -- only 1%.

For the prophylaxis (preventive treatment) and medical treatment of the grippe, use was made of the phytoncides of onion, garlic and dog (hip-bearing) rose. With a medical purpose, phytoncides were employed internally in the form of alcohol-water extracts, counting on 1 drop to 2 kilograms of weight per day. The daily dose was divided into 3 equal parts, and each part, in the form of drops, was used 3 times per day in boiled water. It was established that the effectiveness of medically treating the grippe with phytoncides, in comparison with sulfanilamide preparations, was more pronounced. This was characterized by the absence of subfebrile temperature, by a reduction in the ESR (erythrocyte

sedimentation rate) and leucocytosis, by a reduction in the frequency of complications (4 times in comparison with the action of calcex and 2 times in comparisc with the action of sulfanilamide preparations), and by a better effect on equalizing the water-salt metabolism.

For the prophylaxis (preventive treatment) of the grippe, use was made of garlic phytoncides, and for control, there was taken into consideration the actic of the serum of grippe reconvalescents. The prophylaxis (preventive treatment) was carried out by the intranasal method. The serum and phytoncides were dropped into the nose in an amount of 5 drops twice a day. The medication was carried out for 14 days during the period of the developing grippe outbreak. It was established that a diminution in the prevalence (sick rate) of grippe among those being medicated was noted already after 2 days following the beginning of medication. The positive action gradually increased and yielded a stable effect after 2 weeks following medication. A stable effect, i.e.,non-susceptibility to grippe in both methods of medication, was observed in the course of a month, in spite of the presence of grippe among persons not using medication; in other words, in spite of the presence of contact with grippe patients.

The positive effect of garlic phytoncides manifested itself not only in the intranasal method of administration, but also in the case of interest application of dilutions of 1 : 2000. In such a dilution, the garlic phytoncides were taken in an amount of 1 tablespoon once a day at nighttime (over night) over a period of a month every 4 days with a 4-day interval. It was established that, in a developing outbreak of the grippe, diseases in persons taking garlic were noted to be 4 times less frequent than in those that did not take phytoncides. The act vity of garlic was confirmed by a monocytogram, which characterized the state of the reticulo-endothelial system, which has a significance in raising the capacity to resist the organism. There was noted an increase in the active elements of the mesenchyma (promonocytes and monocytes), and a diminution in the non-active (inactive) elements (polymorphomonocytes). Such a positive reaction was noted in the course of a day following a single internal dose of phytoncides.

An encouraging result was noted also in the action of large dilutions of garlic phytoncides ($10^{\text{minus } 18}$), which were taken internally in an amount of 1 tablespoon once a day over a period of 2 weeks. The effectiveness of such a metl od of prophylaxis (preventive treatment) was characterized by the fact that from a group of patients that did not take garlic phytoncides there were hospitalized 64%, and from a group of patients that took garlic -- only 14%. According to our data, garlic phytoncides in a dilution of 1 : 2000 yield a favorable result in the medical treatment of anemia. In such a dilution the phytoncides were taken internally in an amount of 1 tablespoon twice a day. Even after 10 days of medical treatment there was noted an increase in the hemoglobin by 3% and an increase in erythrocites by 800,000, The positive effect of garlic phytoncides was manifested on the leucocytic formula (there was an increase in the amount of eosinophilia, lymphocytes and monocytes).

A good result was obtained by us as a result of applying the phytoncides of onion, garlic, dog (hip-bearing) rose, century plant, burdock root, spruce and pine needles in the case of suppurative skin diseases. An encouraging result was also obtained in the medical treatment of furunculosis with onion and garlic phytoncides which were employed locally in the form of compresses. It has been established that in medical treatment with onion phytoncides, recovery had its onset after 4 days, with garlic phytoncides -- after 5 days, and in medical treatment with ichthyol -- only after 9 days.

A rather good result was also noted by us in applying garlic phytoncides in sciatica in cases where a long applied physiotherapy and medicinal remedies (drugs) had notyielded any relief.

We also tested garlic phytoncides in chronic colitis and gastritis. In the medical treatment of chronic colitis, a positive result was noted in 70% of the cases, including patients for whom the duration of the disease was equal to 10 years. It is characteristic that in many patients an improvement had its onset as early as the 2nd day of medical treatment, and in a number ofpatients -- after a month of medical treatment. An improvement in the medical treatment of

yperacidic gastritis had its onset after 6 days; this was expressed in an improvement of the overall state of health, in the disappearance of meteorism (flatulence), belching, heartburn, pains in the stomach, in a moderate reduct in the amount of free hydrochloric acid, and in the overall acidity. It is characteristic that such an improvement was noted even in the case of the act of dilutions of $10^{\text{minus } 18}$.

An encouraging result was also obtained by us in the medical treatment o dysentery means of garlic phytoncides in cases where there was an absence of result from the application of other medicinal remedies (drugs). In the overwhelming majority of cases, a daily dose of an alcohol-water extract of garlic calculating on 1 drop to 2 kilograms of the patient's weight, was instrumental in recovery after 3-4 days. It should be noted, however, that in a number of cases, in dysentery of the Flexner type, garlic phytoncides did not manifest any effect, just as other medicinal remedies (drugs).

We have established that garlic phytoncides, even in diluting of 1 : 2000 are instrumental in the quieting of whooping cough in the convulsive stage. It has also been established that the phytoncides of onion and garlic lower t blood pressure. A drop in the blood pressure had an onset even from the act of a dilution of $10^{\text{minus } 6}$.

Proceeding from the data in the literature and from our own observation we consider it expedient to pose a question with regard to the widespread us of phytoncides for prophylaxis (preventive treatment) and for the medical tr ment of many diseases. The discovery of Prof.=Tokin should be incorporated clinical study in spite of an absence of explanatory data with respect to th mechanism of the action of phytoncides.

Address of the author: Gorky, Gruzinskaya Street, No. 46, Apt. 19.

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ANTI-TUBERCULAR ACTIVITY OF GARLIC (*ALLIUM SATIVUM*).

BY

J. C. SURI.

(*From the Central Research Institute, Kasauli.*)

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THERE is a widespread belief amongst the lay public that raw garlic is useful in the prevention and treatment of tuberculosis. This belief has had some measure of support from the medical profession. For instance, Martindale (1941) records that preparations of garlic have been used in the treatment of pulmonary phthisis, bronchiectasis, gangrene of the lung and whooping cough. Poultices of pulped garlic have been recommended for the treatment of lupus and tuberculous ulcers. Inhalations of garlic have also been recommended for bronchiectasis and pulmonary disorders. Garlic is also largely used by practitioners of Ayurvedic and Unani systems of medicine, particularly in early stages of pulmonary tuberculosis.

In view of these popular beliefs, it was decided to test the efficacy of garlic in the prevention and treatment of tuberculosis in experimentally infected animals. Studies were first undertaken to see if garlic had any inhibitory effect on the growth of tubercle bacillus *in vitro*. Two extracts of garlic were used :—

(*a*) *Watery extract.*—Twenty-five grammes of freshly peeled garlic were macerated with 5 c.c. of distilled water. This was strained through fine muslin, centrifuged and the supernatant sterilized by Seitz filtration.

(*b*) *Alcoholic extract.*—Fifty grammes of freshly peeled garlic were macerated with 100 c.c. of absolute alcohol. The alcohol was allowed to act for 24 hours during which time the mixture was frequently shaken. It was strained through fine muslin and then through filter-paper. The filtrate was evaporated in a water-bath at 60°C. till the volume was reduced to 30 c.c. This was stored in the cold storage till required.

Para-amino salicylic acid sodium (PAS), a well-known anti-tubercular drug, was also included in the trials for purposes of comparison.

B. tuberculosis strain No. H52 (human) (National Type Collection Strain) was used as the test organism. This was grown in a modified Proskauer and Beck's medium containing 10 per cent of horse serum. The minimum bacteriostatic concentration of the drugs under test was determined after incubation at 37°C. for 14 days, the first reading being taken on the 7th day. The results are shown in Table I.

TABLE I.

Anti-tubercular action of garlic in vitro.

Concentration of the drug, in g. per cent.	Day of observation.							
	7th.	8th.	9th.	10th.	11th.	12th.	13th.	14th.
Watery extract garlic :								
2·5	—	—	—	—	—	—	—	—
2·0	—	—	—	—	—	—	—	—
1·8	—	—	—	—	—	—	—	—
1·6	—	—	—	—	—	—	—	—
1·4	—	—	—	—	—	—	—	—
1·2	—	—	—	—	—	—	+	+
Alcoholic extract garlic :								
2·4 garlic (in 1·4 alcohol)	—	—	—	—	—	—	—	—
2·0 garlic (in 1·2 alcohol)	—	—	—	—	—	—	—	—
1·8 garlic (in 1·1 alcohol)	—	—	—	—	—	—	—	—
1·6 garlic (in 1·0 alcohol)	—	—	—	—	—	—	—	—
1·5 garlic (in 0·9 alcohol)	—	—	—	—	—	—	—	+
1·4 garlic (in 0·8 alcohol)	—	—	—	—	+	+	+	+
Absolute alcohol (control) :								
2·5	—	—	—	—	—	—	—	—
2·0	—	—	—	—	—	—	—	—
1·6	—	—	—	—	—	—	—	—
1·4	—	—	—	+	+	+	+	+
1·2	—	—	—	+	+	+	+	+
1·1	—	+	+	+	+	+	+	+
Para-amino salicylic acid sodium (control) :								
0·0032	—	—	—	—	—	—	—	—
0·0016	—	—	—	—	—	—	—	—
0·0008	—	—	—	—	—	—	—	—
0·0004	—	—	—	—	—	—	—	—
0·0002	—	—	—	—	—	—	—	—
0·0001	—	—	—	—	—	—	+	+
Inoculum control ...			—	6th day.				
Medium control ...			—	14th day.				

+ = Growth. — = No growth.

ANTI-TUBERCULAR ACTIVITY OF GARLIC *IN VIVO*.

FIG. 1.

PRE-TREATMENT CONTROLS.
(KILLED 7 DAYS AFTER INFECTION.)

TREATMENT COMMENCED 7 DAYS AFTER INFECTION.
GARLIC.

STREPTOMYCIN.

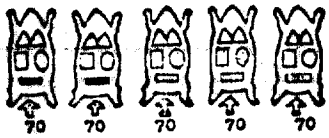
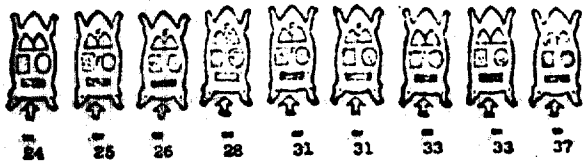


FIG 2.

PRE-TREATMENT CONTROLS.
(KILLED 14 DAYS AFTER INFECTION.)

TREATMENT COMMENCED 14 DAYS AFTER INFECTION.
GARLIC.



STREPTOMYCIN.

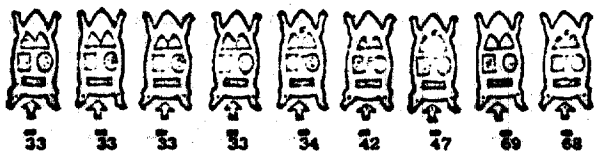
Fig. 3.

PRE-TREATMENT CONTROLS.

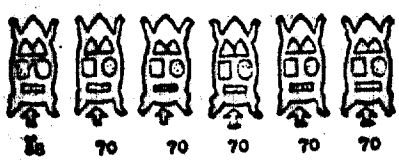
(KILLED 21 DAYS AFTER INFECTION.)

TREATMENT COMMENCED 21 DAYS AFTER INFECTION.

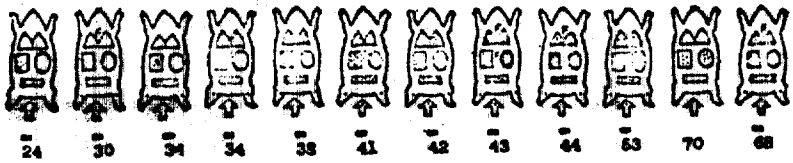
GARLIC.



STREPTOMYCIN.



UNTREATED CONTROLS.



Note.—Extent of tuberculosis seen at necropsy in each animal :—

1. The lungs, liver (▯), spleen (○) and omentum (▭) have been marked. The dot in the arrow represents a nodule at the site of inoculation. Diffuse tuberculous involvement of an organ is indicated by complete blackening. Miliary and nodular lesions are represented by small and large dots, respectively. The numerals indicate the number of days of infection and the black bar indicates that the animal died before the end of the experiment.
2. Three animals died prematurely of intercurrent infection and are not, therefore, included.

From Table I it will be seen that a watery extract of raw garlic exerts bacteriostatic action on the growth of tubercle bacillus in a concentration of 1·4 per cent and alcoholic extract in a concentration of 1·6 per cent. Tuberculostatic action of PAS under the same experimental conditions is of a very much higher order than that of raw garlic.

In vivo experiments were undertaken using guinea-pigs weighing 180 g. to 260 g. Eighty-one animals were divided into three groups: one treated with fresh garlic mixed in their food, the other with streptomycin given subcutaneously and the third group of 21 animals served as untreated controls. Ten animals in each group were treated at 7, 14 and 21 days' intervals after infection. Results are shown in the accompanying diagrams (*see* Figs. 1, 2 and 3). Infecting dose consisted of 0·1 mg. (moist weight) of a 15-day culture of T.B. strain No. H37RV, injected intraperitoneally. The experiment was terminated on the 70th day after infection when surviving animals were killed. Fifty per cent of the animals treated with streptomycin were also killed on the same day to compare the extent of the lesions with those of the garlic group. Post-mortem was done on all animals.

Three guinea-pigs belonging to the control (untreated) group were killed on the 7th, 14th and 21st days after infection to note the progress of the infection.

The results of assessment of lesions in each group are shown in Table II.

It will be seen that the degree of infection in garlic-treated animals is just as great as in untreated controls. The diagrams give in detail the extent of the lesions produced in the treated and untreated animals. For purposes of comparison the results obtained with streptomycin have been included.

Conclusion.

1. Garlic in concentrations varying from 1·4 per cent to 1·6 per cent exerts inhibitory action on the growth of tubercle bacilli *in vitro*.

2. It has no anti-tubercular action *in vivo*. Given every day in a dose of 2 g. of fresh garlic mixed in food to guinea-pigs experimentally infected with tuberculosis, it does not delay or prevent the onset of the disease.

REFERENCE.

Martindale (1941) ... 'The Extra Pharmacopœia', 22nd ed., 1, p. 167.

TABLE II.

Anti-tubercular action of garlic in vivo.

Group.	Treatment started 7 days after infection.			Treatment started 14 days after infection.			Treatment started 21 days after infection.		
	Number died/total number of animals.	Average period to death, days.	Average index of T.B.*	Number died/total number of animals.	Average period to death, days.	Average index of T.B.*	Number died/total number of animals.	Average period to death, days.	Average index of T.B.*
Garlic treated	9/9†	30	7·2	9/9†	30	7·7	9/9†	44	6·8
Streptomycin treated ...	0/10	...	...	0/9	...	...	1/10	58	2·0
Pre-treatment controls (killed on 7, 14 and 21 days after infection).	3	...	0	3	...	1	3	...	2·6
Untreated controls ...	11/12	41	6·7	...	...	...	...	...	...

* According to the amount of gross tuberculous disease, each organ was assigned a value as follows :—

Liver	0 to 4
Lungs	0 to 2
Spleen	0 to 2
Lymph nodes	0 to 1
Omentum	0 to 1
Presence of fluid in chest or abdomen	0 to 1

Maximum total for each animal was, therefore, 12.

Index of tuberculosis is the average total for each group.

† One animal in each group died prematurely of inter-current infection and has been excluded from Table II.

Dose of garlic :—2 g. daily.

Dose of streptomycin :—10 mg. of dihydro-streptomycin sulphate in 2 divided doses morning and evening.

Doklady Akademii Nauk SSSR (Proceedings of the Academy of Sciences of the U.S.S.R.)
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Effect of Phytoncides on the Spermatozoa and Spermatogenesis of Mammals

I.B. Tokin

(Presented by Academician A.I. Oparin on 22 IX 1953 (September 22, 1953)

We were interested in the problem concerned with the effect of phytoncides on the spermatozoa of mammals, and also, in the problem as to whether or not phytoncides, which have been introduced (administered) into the organism, manifest any effect on the normal course of spermatogenesis. Similar investigations, in relation to the phytoncides of higher plants, were not carried out; with respect to the effect of penicillin on the spermatozoa of mammals, and on spermatogenesis, there exists a paper of Molner and Zador (1); the latter have come to the conclusion that penicillin does not manifest any noticeable effect either on spermatozoa (in experiments in vitro) or on spermatogenesis.

1. Effect of phytoncides on the spermatozoa of mammals. We worked with the spermatozoa of guinea pigs and rats. The isolated epididymis of a sexually mature male was placed in a salt cellar with 4 cubic centimeters of a physiological solution and was cut up into small pieces. After the emerged spermatozoa had formed a uniform suspension, they were used for the experiment. An investigation was of the effect of volatile fractions of phytoncides and of phytoncides of the tissue juices (saps) of a number of plants. In studying the action of the volatile fractions of phytoncides, use was made of the method of a suspended drop, repeatedly described in the literature on phytoncides (2). The dimensions of the experimental vessel were 31 X 4 millimeters. The source of the phytoncides was 0.9 gram of the plant material.

The results of the experiments on the spermatozoa of the rat and of the guinea pig are set forth in Table 1.

((Table on pg. 567 of text))

Table 1

Summarized Results of Experiments on the Effect of Volatile Fractions of Phytoncides on the Spermatozoa of a Rat and of a Guinea Pig

Plants whose Phytoncides were Used	Parts of Plants Used	Time Prior to the Stoppage of Movement of Spermatozoa, in minutes	
		Rat	Guinea Pig
Onion	Bulb (Head....	1.5	1.5
Garlic	"	5.5	4.5
Cherry Laurel	Leaves	4.5	4.5
Mustard	Seeds	1.5	1.0
Bird Cherry	Buds	5.5	5.5
Carrot	)		
Garden Beet	) Root	Not active	Not active
Radish	)		
Lemon	Leaves	"	"

((text continued)): We made a study of the "dying morphology" of spermatozoa. It was disclosed that outwardly, even in the case of very high microscopic magnifications, no changes were revealed. In staining the spermatozoa that had been killed by the phytoncides (with iron hematoxylin, according to Geidengain, with a Biondi mixture and with safranine-licht gruen (light green), we could not disclose their distinction from the stained control spermatozoids which had not been subjected to the action of phytoncides (OsO_4 was employed in the capacity of a fixative).

A small detail is of interest: if one should observe spermatozoa that had died without any influence, it will seem that after some time, there takes place a disintegration of the spermatozoa in the region of the neck, and the head is separated from the tail; after tha, the head also disintegrates. This is not observed in spermatozoa treated with phytoncides. This phenomenon may approximately be explained by the fact that the neck of the spermatozoa turns out to be the most vulnerable place for bacteria, and the phytoncides, as antiseptics, hamper the disintegrating action of the bacteria. In favor of this is also the fact that the rat spermatozoa, which have been treated by volatile phytoncides, and which have been kept, over a period of 2.5 years, in a hermetically sealed vessel (in a suspended drop in a physiological solution), up to this time show no

morphological distinction from normal ones.

We studied the effect of the tissue juices (saps) of a number of plants. A drop of a physiological solution with spermatozoa was joined with a drop, having the same volume, of the plant juice (sap) being tested. It was made clear that the juices of garlic, onion and cornel (cornelian cherry) instantaneously stop the movement of the spermatozoa, thejuice of the garden beet -- after 1.5 minutes, that of the radish -- after 12 minutes, and that of carrots -- after 18 minutes. Extractions of the cherry laurel (dilution in a physiological solution of not more than 1 : 5) are not active.

(Data obtained during work with rat spermatozoa).

2. Effect of phytoncides on the spermatogenesis of mammals. We were convinced that the phytoncides of a number of plants that we had studied, including alimentary (nutritive) ones and those employed by medicine, manifest a strong toxic action on spermatozoids under conditions of in vitro experiments. On the other hand, it has been proved that in inhalations of the volatile phytoncides of ionion and garlic, some of their components appear instantly in the blood lumen, and it is as if there were no barriers for them. We carried out an investigation of the problem concerned with the possible effect of phytoncides on the spermatogenesis of white rats.

A mature male rat was placed in an exsiccator having a volume of 2200 cubic centimeters; into it were introduced 20 grams of freshly-prepared pulp (on a grater) from a garlic bulb (head). The edges of the exsiccator were lubricated with vaseline. After 1.5 hours the rat was liberated from the exsiccator. Such inhalations were carried out daily over the course of a week; following this, the testes were removed and were subjected to histological study. In another series of experiments, the garlic juice, in a dilution of 1 : 1, was administered daily, per os, in an amount of 2 cubic centimeters. The experiments lasted for 24 days; following this, the testes were removed and subjected to histological study. Finally, experiments were carried out with the endotracheal administration of garlic juice (dilution of 1 : 5). The juice was administered in an

amount of 0.8 cubic centimeter, daily, over the course of a week.

The results of the histological studies were the same in all cases. The material was fixed with a Tsenker mixture, and it was stained with hematoxylin, according to Geidengain. We may confirm, on the basis of a painstaking microscopic study, that the process of spermatogenesis is normal. We set up additional experiments that convinced us of the fact that neither the production of sexual products, nor the impregnating (fertilizing) capacity of the spermatozoa were impaired. The male, to whom, for several days, there were administered phytoncides, was mated with a female. In order to eliminate the possibility of impregnation with spermatozoa that were located in the epididymis prior to the administration of the phytoncides, the very same male was mated, 2 - 3 times, with new females. Impregnation processes occured in all cases and a normal pregnancy was ascertained.

The A. A. Zhdanov Leningrad State University

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