soybean contained a substance which greatly reduced the activity of trypsin *in vitro*. Ham, *et al.* (*7*) found that a solution of the partially purified inhibitor produced lowering of growth in chicks fed on a well-balanced diet. In a previous communication from this laboratory (*3*) it was stated that glycinin is more nutritive than autoclaved soybean. It was believed that the enhanced nutritive value of glycinin was due to elimination of the proteolytic inhibitor, as also any toxic factor which may be present in raw soybean.

The present investigation was undertaken to see whether the presence of the inhibitor would affect the nutritive value of soybean protein digested with enzyme so that the amino acids are in free form. According to the hypothesis of Melnick, *et al.*, the biological value of the digest is expected to be higher than the raw bean, because all the amino acids, including methionine will be simultaneously available for utilization.

The digest was prepared by subjecting soybean meal to digestion by papain. Acid hydrolysis was not resorted to because it would destroy tryptophane and other possible essential peptide factors like strepogenin, as suggested by Woolley (*5*). Papain was chosen for the proteolytic digestion, since the raw soybean meal contained the tryptic inhibitor. The soybean meal was finely ground and suspended in about four times its weight of water and digested with an active preparation of papain. The digestion was carried out at 50°C. for 48 hours. A control was run, using a boiled solution of the enzyme which was maintained at the same temperature. Both samples were then dried in a current of warm air. It was found that the inhibitor was destroyed to a small extent in the course of drying. A requisite amount of the inhibitor solution extracted from raw bean was added to both the extracts in order to restore the original activity.

The biological value and the digestibility coefficient of the protein in the digested and control samples were determined by the usual nitrogen balance method. The proteins were fed at a level of 10 per cent (nitrogen × 5.7). The results are as follows:

| | Average digestibility coefficient | Average biological value |
|---|---|---|
| Digested sample | 89.6 | 45.9 |
| Control sample | 89.2 | 44.7 |

The nitrogen in the digested sample was analyzed, and it was found that 91.6 per cent of the total nitrogen of the meal was in the nonprotein form, as estimated by its solubility in 7 per cent trichloroacetic acid, and 90 per cent of the total methionine of the protein was in the free form in the digest.

From the above results it is evident that the role of the proteolytic inhibitor in deciding the nutritive value of raw soybean is not in diminishing the degree of availability or rate of release of methionine. It is highly probable that its action is of the nature of an antigrowth factor affecting the usefulness of proteins in general. This statement is supported by the findings of Ham, *et al.* that a purified solution of proteolytic inhibitor caused growth retardation in chicks fed on a well-balanced diet.

It is thought that, apart from the tryptic inhibitor, there may be other toxic factors associated with the raw bean which are destroyed on heating. Evidence for the existence of a separate factor apart from the proteolytic inhibitor was adduced from the following experiment. Everson, *et al.* found that the growth-promoting value of soybean protein increases to a marked extent if the bean is germinated. This observation was also confirmed here, and on examining the concentration of the proteolytic inhibitor in germinated soybean (48 hours) it was found that it was not altered from that in the original bean. The concept of the proteolytic inhibitor cannot explain this increase in nutritive value after germination. That there was no change in the amino-acid composition of the protein after germination was proved by Block, *et al.* (*2*), who could detect no change in the contents of tyrosine, tryptophane, phenylalanine, cystine, and methionine in the protein of soybean after germination.

From the above findings it can be postulated that apart from the proteolytic inhibitor there is a separate factor which affects the nutritive value of the soybean protein. Further work on the isolation of this factor and the respective roles of these inhibitors in the nutritive value of soybean is in progress.

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## Effect of Benzene Hexachloride on the Flavor of Poultry Meat


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Tests conducted during the summer of 1947 show that the meat of chickens fed, sprayed, or retained in a house previously sprayed with benzene hexachloride has a distasteful flavor. This flavor, which makes the meat nearly inedible, persists for a period of 6–10 weeks.

The absorption of a benzene hexachloride flavor and odor was first discovered accidentally. In the fall of 1946 and spring of 1947 a number of chemicals were tested for the control of chicken mites. A paper on the results of these tests is in press for the *Journal of Economic Entomology*. During the course of this study several chemicals, including benzene hexachloride, showed promise, and a test to determine their toxicity to poultry was undertaken. A 5 per cent concentration of wettable benzene hexachloride containing 5 per cent of the $\gamma$ isomer was applied to several chickens, the house, and the litter in December 1946. This test was concluded, and the house was not cleaned up or used until May 1947. At this time market birds were placed in the house and fed grain in the litter as well as mash in a feeder for a period of 1 week. When cooked, the meat of these birds was not edible because of a distasteful flavor apparently caused by the absorption of benzene hexachloride.

Since many manufacturers and promoters of insecticides had used and are now recommending benzene hexachloride

[1] With the cooperation of the Departments of Poultry Husbandry and Home Economics.

in poultry houses, it was decided that a determination of the manner of absorption and persistence of the benzene hexachloride flavor should be made. To this end the following test was set up. Four chicken houses were selected. In one house, 5 birds were fed for 1 week with a mixture of 1 part of benzene hexachloride containing 6 per cent of the $\gamma$ isomer in 200 parts of a mash-grain feed, and then transferred to the standard ration received by the other lots. In the second house, 5 birds were sprayed until wet with a benzene hexachloride suspension containing 0.25 per cent of the $\gamma$ isomer. In the third, the walls were sprayed until wet with a benzene hexachloride suspension containing 0.25 per cent of the $\gamma$ isomer and allowed to dry before the birds were placed inside. In the fourth house, which was isolated from the treated lots, 5 untreated birds were retained as controls. Sprays were applied with a 3-gallon compressed-air sprayer. The birds used were 2-pound White Leghorn cockerels.

One of the birds in the lot receiving the treated feed died at the end of the first week. The bird apparently died of starvation, since the entire lot ate less than a pound of the treated feed in the 7-day period. One of the birds in the sprayed lot also died. These deaths reduced the samples from the 5 originally planned to 4. The remaining birds remained healthy until sampled and killed. The test was begun on June 13, 1947, and the final sample was taken on August 22, 1947.

TABLE 1

COMPARATIVE ABSORPTION AND RETENTION OF BENZENE HEXACHLORIDE ODOR AND TASTE BY CHICKENS

| Treatments | | Comparative rating of odor and taste | | | |
|---|---|---|---|---|---|
| | | 2 weeks | 4 weeks | 6 weeks | 10 weeks |
| Feed treated | Odor | Slight | Moderate | Strong | Strong |
| | Taste | Slight | Moderate | Strong | Strong |
| Birds sprayed | Odor | Moderate | Strong | Strong | Moderate |
| | Taste | Strong | Strong | Strong | Slight |
| House sprayed | Odor | Strong | Slight | Slight | Slight |
| | Taste | Moderate | Slight | Slight | Moderate |
| Control | Odor | Not checked | None | Slight | None |
| | Taste | Not checked | None | None | None |

The first 3 samples were taken at intervals of 2 weeks and the last after a 4-week interval. At sampling time 1 bird from each of the lots was killed and cooled. The following day the birds were roasted in separate ovens at uniform temperatures, no salt or other condiment being used. Five tasters or samplers then checked each bird, which was identified only by a code letter. Checks were made by smelling each of the partially carved carcasses and then tasting small pieces of white and dark meat cut from each bird. Each sampler recorded his own reaction to the quality of the particular meat.

The terms used to describe odor were very strong, strong, sharp, bad, slight, questionable and good; those used to describe taste were very strong, strong, acidic, musty, medicinal, biting, objectional, and good. The terms were modified into those used in Table 1, which gives the results of the test. Statements concerning the presence and comparative degree of an off-flavor or odor are included in the table only when the reactions of 3 or more of the samplers were the same.

# *In Vitro* Resistance of the Genus *Bacteroides* to Streptomycin

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There is little reference to the genus *Bacteroides* in the growing literature on the *in vitro* sensitivity of various gram-negative bacteria to streptomycin. Since these microorganisms, normally saprophytic inhabitants of the nasopharynx (*16*), gastrointestinal tract (*11*), and the female genitalia (*13*), frequently invade the deeper tissues of the body to produce serious and often fatal disease in man (*19, 20*), it seemed worth while to determine their sensitivity to streptomycin.

The obligate anaerobic, nonsporulating gram-negative bacilli comprising the genus *Bacteroides* are a heterologous group closely related to the genera *Dialister* and *Fusobacterium*. Bergey (*1*) lists 22 species of this genus, but since these have not yet been studied systematically, it is impossible to determine how many of them actually represent distinct species. Weiss and Rettger (*21*), for example, were able to classify 73 different strains into 4 serologic groups. Henthorne, Thompson, and Beaver (*14*) have reported that pleomorphic strains are serologically distinct from the nonpleomorphic strains. Therefore, following the convention adopted by Smith and Ropes (*20*), the strains reported in this study were classified as *B. funduliformis* if pleomorphic and *B. fragilis* if nonpleomorphic. Pleomorphism was determined by the formation of large bodies and filaments in artificial media and by a form of reproduction different than simple binary fission, associated with the production of anaerobic pleuropneumonia-like colonies (L-variation), as described by Dienes (*6*) and Dienes and Smith (*7–9*).

Four strains of *B. funduliformis* and 8 of *B. fragilis* have been encountered in this laboratory during the past year. These were isolated in primary culture on anaerobic horse blood or ascitic fluid agar plates, or in sodium thioglycollate broth. Subcultures were carried in chopped meat or sodium thioglycollate enriched with 30 per cent ascitic fluid and sealed with vaseline. All strains produced varying amounts of gas in these media and were characterized by a sharp, acrid odor. Strains were examined for pleomorphism and the production of anaerobic pleuropneumonia-like colonies by the cultivation and in situ agar block staining methods previously described by Dienes (*5*).

Streptomycin sensitivity was determined in unsealed 0.1 per cent sodium thioglycollate broth containing 0.1 per cent agar-agar, according to a titration based on the methods described by Price, Nielsen, and Welch (*17*). Streptomycin sulfate was diluted so that 4 times the desired doses were contained in 0.5-ml. volumes of broth. To these 0.5-ml. volumes was added 1.5 ml. of a 1:100 dilution of an 18- to 24-hour culture of the strain to be tested, giving a titration with final concentrations of streptomycin ranging from 4 0 mg. to 2.0 $\mu$g./ml. in a total volume of 2.0 ml. At the same time, each strain was tested for its ability to grow in the presence of 100 Oxford units of penicillin/ml. in the same medium. All titrations were examined after 48 hours incubation at 37°C. The first tube in which growth was inhibited was taken as the minimal inhibiting dose.

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