AN EXPERIMENTAL STUDY ON MALT-SPROUT POISONING.*

USHIO TANAKA.

(From the Veterinary Laboratory, Ministry of Agriculture and Commerce, Nishigahara, Tokyo, Prof. Dr. N. Nitta, Director.)

Introduction.

Malt-sprout, a by-product of beer-brewing, is often used as a fodder for domestic animals, especially for cattle and pig, as it contains not less nutritious matter than other usual fodders, but we must always bear in mind that the animal should not be allowed to take it excessively because it contains even in fresh state some poisonous ingredients, maximal quantity for a cow being 1–2 kilos per day.

In December, 1918, a number of cases of forage poisoning occurred in a dairy in Tokyo and twenty cattle were taken ill, five of them being dead. Prof. G. Suto, who was called to examine the sick animals, declared that the cause of the poisoning should be attributed to the malt-sprout, which was given to the animals too much on that occasion. S. Itagaki, who assisted to examine the fodder, told me that the fodder was found in a very rottened state, smelling unpleasant and disagreeable in appearance. According to their explanations, I could acknowledge among the symptoms manifested by the sick animals two characteristic features, one of which being a very sensible state to an external impulse and another the symptom of inflammation in the stomach and intestine.

From the above symptoms it may be considered that the disease was caused by some poisonous products due to the putrefaction of malt-sprout or by some poisonous ingredients naturally contained in it.

In order to determine the principal poisonous substance contained in the fresh malt-sprout and whether it would produce similar symptoms noted in the actual cases, I carried out some experiments on laboratory animals. The following is a brief description of these experiments.

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Chemistry of Malt-Sprout.

Malt-sprout, younger root of barley, is yellowish brown in color and has a pleasant and scorching smell. Its known ingredients are as follows:

1. Betain and Cholin; 2. Saccharose; 3. Alkaloid Hordenine; the amount of it being 0.2%, its toxicity very feeble; 4. Amide in a large quantity.

The chemical constitution of the malt-sprout is: Water 12%, Nitrogenous matter 23%, Pure protein 16.28%, Fat 2%, Crude fiber 12.32%, Nitrogen-free extractive matter 4.3%, Ash 7.51%, potassium and silica being much in ash.

Notes on the Experiments.

As a first step, I have carried out some experiments in order to determine whether the clinical symptoms could be produced in experimental animals by feeding on fresh malt-sprout. With regard to this investigation I have not experimented on larger animals owing to the want of supply of animals and materials. S. Kondo of this laboratory has, however, already in 1919 made an experiment on this subject using two calves. According to him the principal symptoms of intoxication caused by the sprout in cattle are to be noticed especially in the nervous system. The affected animals became very nervous, being apt to be irritated by a slight external impulse. This nervous symptom resembled very much that noticed in the actual cases mentioned above. And other symptoms noted in those cases might probably be caused by some poisonous matters due to the putrefaction of malt-sprout.

In my feeding experiments on rabbits and guinea-pigs, which have been allowed access to the malt-sprout, for example, one-tenth to one-fifth of body weight, I could find no symptoms of intoxication during the course of experiment. The animals were very healthy and their weight increased gradually. From these results, it would be concluded that the malt-sprout had no harmful effect on animals which were fed with it, that means, a certain quantity of the malt-sprout could be given as an auxiliary
fodder to domestic animals, if added to other principal fodders as barley, oat, etc.

With the view of examining the toxicity of the malt-sprout, I have made further experiments in such a way that in the form of watery and alcoholic extracts the sprout was given per os to animals in greater quantity at one time than in the case of the feeding experiment above mentioned. The extracts employed in these experiments were prepared in the following way:

**Watery Extract:** A mixture of 500 grams of air-dried malt-sprout and 4 liters of water is placed in a room, allowing it to stand in the open air at night for the purpose of preventing fermentation. After two days, this mixture is filtered with cotton cloth, together with its pressed fluid and the dark brown, turbid filtrate thus obtained, showing weak acid reaction, is then submitted to vacuum distillation below 50°C. and 15 mm Hg. The resulting extract, amounting to 950 grams, may be administered per os to rabbit.

**Alcoholic Extract:** To 500 grams of the malt-sprout 3 liters of spiritus (Ph. J. IV.) are added and the mixture is allowed to stand for two days, being stirred up from time to time. After filtering, the alcoholic extract is condensed, with its pressed fluid, at first to 150 c.c. below 40°C and 15 mm Hg. and then to the weight of 24 grams on water bath. At last it changes to a viscid mass which is acid in reaction and dark brown in color. When administering this extract to rabbits, it is diluted to 10 c.c. with distilled water.

Employing these extracts, I have made some experiments on rabbits to which 50 to 100 or 150 grams of malt-sprout per 1 kilo body weight were given at one time, the amount being exceedingly greater as compared with that of the feeding experiment above mentioned. But contrary to my expectation, the results were negative, the animals which ingested the extract manifesting no symptoms of intoxication.

From these results, I am inclined to believe that the malt-sprout does not always cause the intoxication in animals, if given under suitable attention.

Finally, I carried out some injection experiment employing
known ingredients of the malt-sprout. As above mentioned, the malt-sprout contains many basic compounds which are produced by special metabolism, especially during the course of germination of the seed. But among its constituents, at the same time, there are always to be found their antagonists i.e. substances of acid reaction. Therefore, before examining the basic ingredient, I extracted all the acid substances in the following manner: after treating the material three times with alcohol acidified by the addition of hydrochloric acid, the transparent filtrate is neutralized by the addition of sodium carbonate and condensed to one-tenth of its original volume and the residual alcohol is again evaporated on water bath. Then the condensed liquid is shaken five times with ether, after adding to it hydrochloric acid in a proportion of 3%, and when ether is evaporated, a dark brown extract is obtained, amounting to 0.1% of the malt-sprout. After neutralizing this acid substance with sodium carbonate, and making a 20% solution by dissolving it in physiological salt solution, I injected the solution in various quantities into rabbits and guinea-pigs. These injection experiments, however, gave no positive results.

The failure of producing symptoms of intoxication in the experimental animals has caused me to examine the basic constituents of the malt-sprout. Among its ingredients I think hordenine will most likely be the actual exciting cause of intoxication in animals.

Hordenine, p-oxyphenyldimethylethylamine, was first discovered by Léger in the malt-sprout, which contains 0.2% of it and it forms colorless needles fusing at 117.8°C. He succeeded in making its sulphate, hydrochlorate and hydrobromate, determining its chemical constitution. Berger, Ehrlich, Rosemund, and Voswinckel ascertained later that its formula is quite correct and consequently Léger's statement is true.

The action of hordenine on the animal body has hitherto not been studied exactly. Hordenine sulphate is a very weak poison, affecting chiefly the nervous system, the cause of death being paralysis of respiration. Animals receiving the toxic dose, less than fatal, generally recover in a short time (Camus). The lethal dose of hordenine sulphate for dog is 0.3 grams per kilo when
administered intravenously and that for guinea pig 2 grams per kilo when administered subcutaneously. Therefore, its toxicity is very weak. When a cat is injected intravenously with 1 gram of its methyl iodate, a temporary rise of blood pressure takes place, as in the case of adrenalin injection, but its mode of action belongs to the type of the group of nicotine.

Preparation of hordenine. I have prepared hordenine from malt-sprout according to G. Otto Gaebel's method with occasional suitable modifications. 3 kilos of fresh malt-sprout received from a beer-brewery are extracted with 94-96% alcohol in a flask provided with a reflux condenser for 35 hours and this procedure is repeated twice more, renewing the alcohol (according to Gaebel the most suitable solvent is 96% alcohol).

After filtrating, the hot alcoholic solution is allowed to stand over night to remove impurities. The alcohol is evaporated on water bath till the solution becomes somewhat viscid and to it 1 liter of water is added to cause the precipitation of residual impurities. The extract thus obtained is alkalized by adding anhydrous potassium carbonate and then shaken with one-half volume of dried ether twelve times.

Thus hordenine remove to ether. After drying by the addition of some potassium carbonate the solution is distilled and the impure hordenine is found in crystalline form. By submitting the impure hordenine to careful recrystallization two or three times, making use of dried ether, we can easily obtain beautiful needles of alkaloid hordenine which amounts to about 9 grams, corresponding to 0,3% of original malt-sprout. These colorless needles fuse at 117-118°C, dissolve in water and ether with difficulty, reacting weak alkaline. When some concentrated hydrochloric acid is added to these crystals, the latter immediately change into very fine needles of hordenine hydrochlorate. In the injection experiment I used always this salt dissolved in the physiological salt solution.

Symptoms produced by injecting hordenine. The following characteristic symptoms have been observed in mice, guinea-pigs, and rabbits into which a certain quantity of hordenine hydrochlorate was injected intravenously or intraperitoneally.
Mouse: in a few minutes after the intraperitoneal injection of hordenine it manifests retarded respiration, and at the same time trembles severely, followed by marked dyspnea. Then it remains lying, being unable to rise. Death is preceded by a struggle of agony. The lethal dose is 0.4 gram of hordenine per 1 kilo body weight.

Guinea-pig: the symptoms are especially characteristic; after the intravenous injection its behavior suddenly changes and a great exaltation is to be seen; it runs out of tray, its nose being collided with covering net, causing haemorrhage, or by a slight physical stimulation it becomes mad, showing a strong excitement of nervous system. If a large dose is injected, death takes place immediately under strong dyspnea and agony. By administering a comparatively small dose we can observe the characteristic symptoms. The fatal dose for guinea-pig is 0.3 gram per kilo.

Rabbit: hordenine does not act upon it so markedly as in the case of guinea-pig. When injected intravenously it becomes very timid, trembling by a very slight stimulation. In the administration of a larger dose it falls down to death, showing severe agony. The lethal dose is 0.2 gram per 1 kilo.

**Summary.**

1. The disturbances caused by ingestion of malt-sprout are to be attributed to the action of hordenine, an alkaloid naturally contained in it, but the toxicity of this alkaloid is very weak.

2. It will not be always irrational to utilise the malt-sprout under suitable attention as an auxiliary fodder, comparing its weak taxicity with the nutritious value of it.

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むぎめ中毒ノ実験的研究
田 中 亜 雄
（農商務省畜疫調査所）

著者ハ大正7年藤穂東京帝國大學教授ニヨリ発表セラレタル畜牛＝於ケルむぎめノ中毒＝関スル報告＝基キ各種実験動物（家兎もるもっこ及二十日鶏＝＝就キ新鮮ナルむぎめヲ用ヒテ中毒試験ヲ行ヒタリ単ヲ先づ2箇月餘＝互リテ著シキ大量ノむぎめヲ単＝次デ水性及あるこー＝性越幾＝ニヨリ更ラ＝多量ノむぎめヲ1回＝與へ其中毒症狀ノ発現ヲ期シタリ然ル＝其結果ハ總ヲ陰性＝終レリ最後＝鰈基性物質ト酸性物質トノ抽出ヲ行ヒテレヲ直接試験動物ノ静脈内及腹腔内＝注入シタル＝酸性物質＝依リテハ何等ノ中毒ヲ見ザリシ＝鰈基性物質中ハる＝於ニヨリテ実例ト一致スベキ成績ヲ挙グルコトヲ得タリ但其用量ノ顕ル－多量ナル＝諦ミ其毒性ノ著シク薄弱ナルノ知ル＝得タリ＝是＝等ノ試験＝結果著者＝むぎめハ新鮮＝シテ過量ナルザル＝於ハ主ナル家畜飼料＝對ノ補助的ノ飼料トシテ必要シメ排斥スペキモノ＝非ストナス＝至レリ

（自抄）