THE CHEMISTRY OF TYROTOXICON, AND ITS ACTION UPON LOWER
ANIMALS.*

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SINCE making my last report on the investigations concerning the nature of tyrotoxinon, I have continued my work, aided greatly by Messrs. F. G. Novy and E. V. Riker. We

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soon ascertained that if some butyric acid ferment be prepared as is ordinarily done in the preparation of butyric acid, and some of this be added to normal milk, and the whole be kept in closely stoppered bottles for eight or ten days, the poison will be developed in the milk in considerable quantity. The milk should be filtered, the filtrate neutralised with sodium carbonate, and then extracted with ether.

Having a strong solution of the poison in absolute alcohol, which had been obtained from milk inoculated as stated above, we added to it some platinum chloride, and began to evaporate on the water-bath. As soon as the alcohol evaporated the residue exploded with great violence. The experiment was repeated a number of times with like results. From some of this alcoholic solution the platinum was removed with hydrogen sulphide gas; but the filtrate was then found to have lost its explosive property. This reminded us that diazobenzol compounds form with platinum chloride a highly explosive salt, and that diazobenzol is also decomposed by hydrogen sulphide.

Some diazobenzol was prepared according to the method of Griess (*Annalen der Chemie und der Pharmacie*, vol. 137), and comparisons made between this and tyrotoxin. With equal parts of sulphuric acid and carbolic acid the prepared diazobenzol nitrate gave a green coloration, while with the same reagents tyrotoxin gave a colour which varied from a yellow to an orange-red. But the diazobenzol nitrate dissolved in the whey of normal milk, and extracted with ether, or in the presence of other proteids, gave the same shades of colour as the tyrotoxin did, and the potassium compound of the tyrotoxin, prepared by the method to be given later, gives the green coloration. This colour test may be used as a preliminary test in examining milk for tyrotoxin. It is best carried out as follows:—Place on a clean porcelain surface two or three drops each of pure sulphuric acid and pure carbolic acid. This mixture should remain colourless or nearly so. Then add a few drops of the aqueous solution of the residue left after the spontaneous evaporation of the ether. If tyrotoxin be present an orange-red to a purple colour will be produced. This test is to be regarded as only a preliminary one, for it may be due to the presence of a nitrate or nitrite. The tyrotoxin must be purified according to a method to be given farther on before the absence of a nitrate or nitrite can be positively demonstrated.

In the filtrate from milk which is rich in tyrotoxin, after neutralization with sodium carbonate, filtration, and acidifying with hydrochloric acid, gold chloride produces a precipitate, which is insoluble in water, but soluble in hot alcohol, from which it separates on cooling in golden plates. Diazobenzol gives with gold chloride a precipitate having all these properties. In both cases the gold compound is decomposed by frequent treatment with hot alcohol, and this fact prevented any satisfactory ultimate analysis of this compound. It should be remarked here that from some samples of milk this gold salt is obtained much more easily than from others, and the difference is dependent not so much upon the amount of tyrotoxin present, as upon the condition of the other organic matter present. It is best obtained from samples which have stood in well stoppered bottles for a month or longer.

Thinking it not likely that the diazobenzol existed in the cheese and milk as a nitrate, we prepared some diazobenzol butyrate, and found the crystals of these to agree exactly with those of tyrotoxin, and that they decomposed with like rapidity when exposed to moist air.

From tyrotoxinon obtained from milk, diazobenzol-potassium hydrate was obtained according to the method of Griess, and the per cent. of potassium in this compound was determined. The filtrate from the milk, which had been inoculated with the ferment, and kept in a stoppered bottle in a warm room for ten days, was neutralized with sodium carbonate, agitated with an equal volume of absolute ether, allowed to stand in a stoppered flask for twenty-four hours, the ether removed and allowed to evaporate from an open dish. The aqueous residue was acidified with nitric acid, then treated with an equal volume of a saturated solution of potassium hydrate, and the whole concentrated on the water-bath. On being heated the mixture became yellowish-brown, and emitted a peculiar aromatic odour. Both the colour and odour corresponded exactly with the colour and odour produced by carrying some of the artificial diazobenzol through a comparative test. On cooling the mass crystallised, the diazobenzol-potassium hydrate appearing in the test with the tyrotoxinon, and in the comparative test also, in beautiful six-sided plates, along with the prisms of potassium nitrate. The crystalline mass was treated with absolute alcohol, filtered, the filtrate evaporated on the water-bath, the residue dissolved in absolute alcohol, from which the diazobenzol-potassium hydrate was precipitated with ether. The precipitate was collected, washed with ether, dried, and the per cent. of potassium estimated as potassium sulphate. 0.2045 gram. of the diazobenzol-potassium hydrate yielded 0.109 gram. of potassium sulphate. Per cent. of potassium calculated, 24.42; found, 23.92.

Chemists will now appreciate the great difficulty that has been experienced in isolating the active agent of poisonous cheese. The readiness with which diazobenzol decomposes is well known. When warmed with water it breaks up into carbolic acid and nitrogen. Hydrogen sulphide decomposes it; therefore all attempts to obtain the poison by precipitating it with some base, such as mercury or lead, and then removing the base with hydrogen sulphide, have failed. Moreover, diazobenzol is only a transition product of putrefaction. I have frequently found that leaving some milk rich in the poison in an open beaker for twenty-four hours would be sufficient to destroy the whole of the poison.

The following experiments will show that the effects of tyrotoxinon and diazobenzol upon the lower animals are identical:—

Experiment 1.—From one half gallon of some milk which had stood in a tightly stoppered bottle for three months, there was obtained quite a concentrated aqueous solution of the poison, after the spontaneous evaporation of the ether. Ten drops of this placed in the mouth of a small dog three weeks old caused within a few minutes frothing at the mouth, retching, the vomiting of frothy fluid, rapid breathing, muscular spasm over the abdomen, and, after some time, watery stools. The next day the dog seemed to have partially recovered, but was unable to retain any food. This condition continuing for two days, the animal was killed with chloroform. No examination of the stomach was made.

Experiment 2.—Tyrotoxinon obtained from poisonous ice-cream was given to a cat. Within ten minutes the cat began to retch, and soon it vomited. The retching and vomiting continued for two hours, during which time the animal was under observation, and the next morning it was observed that the cat had passed several watery stools.

After this, although the cat could walk about the room, it was unable to retain any food. Several times it was seen to lap a little milk, but on doing so it would immediately begin to retch and vomit. This condition continuing, after three days the animal was placed under ether, and its abdominal organs examined. We certainly expected to find marked inflammation of the stomach; but we really did find the stomach and small intestines filled with a frothy serous fluid, such as had formed the vomited matter, and the mucous membrane very white and soft. There was not the slightest redness anywhere along the alimentary canal.

Experiment 3.—Some tyrotoxin obtained from milk which had been inoculated with poisonous cream, and allowed to stand for forty-eight hours, was administered to a large old cat. It soon produced retching, but no vomiting or diarrhoea. The amount of the poison administered in this case was small.

Experiment 4.—Some tyrotoxin from milk was given to a young, but full-grown cat. Within fifteen minutes there was marked and evidently painful retching, and within half an hour vomiting accompanied by rapid breathing. Later there were several stools, the first two of which contained fecal matter; but subsequent ones were rice-water like, and wholly free from fecal odour. After two days some more of the poison was given, and the vomiting and diarrhoea again induced. The animal was then anaesthetized, and examination of the stomach and intestine showed the mucous membrane blanched, as was found in experiment 2.

We have the records of a number of other experiments with tyrotoxin on the lower animals; but as the symptoms induced in all were substantially the same, it is unnecessary to note them here. We will now give the effects observed in the lower animals after the use of the prepared diazobenzol.

Experiment 5.—Gave to a large old cat 100 milligrams. of diazobenzol butyrate. Immediately the animal began to purge. Then she lay upon the floor breathing rapidly and retching severely for two hours, when she died. The retching was most violent, but vomiting seemed impossible. *Post-mortem* examination showed the lungs greatly congested, but the mucous membrane of the stomach and intestine was not reddened. The stomach contained some food. I suppose that the congestion of the lungs was due to the violent retching.

Experiment 6.—To a young, but full-grown Maltese cat I gave 100 milligrams. of diazobenzol butyrate. With most violent retching, but without either vomiting or stool, the animal died within thirty minutes after the administration of the poison. The lungs were found acutely congested, and the stomach free from any redness. The circular fibres of the small intestine were tightly contracted.

Experiment 7.—Gave to a full-grown cat 25 milligrams. of diazobenzol butyrate. Within ten minutes vomiting and purging were induced. The first stools contained fecal matter; but the subsequent ones were like rice-water, and wholly free from fecal odour. After two days the cat was able to take food; then 10 milligrams. more of the poison were given, with the reproduction of the vomiting and purging. The animal then rapidly emaciated, and after a few days it was anaesthetised, and the mucous membrane of the stomach and intestine found blanched. The lungs were not congested.

Experiment 8.—10 milligrams. of the poison produced profuse diarrhoea, and continued vomiting in a cat.

Experiment 9.—75 milligrams. produced vomiting and diarrhœa with congestion of the lungs in a dog.

It seems unnecessary to detail any more of these experiments, as the identity of tyrotoxin with diazobenzol is now established, not only by chemical analysis, but this proof is strengthened, if chemical analysis can be strengthened, by the action of the poison on the lower animals, and by the *post-mortem* appearance.

I think it highly probable that diazobenzol or some closely-allied substance will be found in all those foods which from putrefactive changes produce nausea, vomiting and diarrhœa. In some oysters which produced these symptoms I have recently found tyrotoxin.

Milk or other fluid to be tested for this poison should be kept in well stoppered bottles; for if the fluid be exposed to the air the tyrotoxin may decompose in a few hours. The filtrate from the milk, or the filtered aqueous extract of cheese should be neutralised with sodium carbonate, then shaken with half its volume of pure ether. Time should be given for the complete separation of the ether. Purified tyrotoxin is insoluble in ether, and it probably owes its solubility in ether at this stage to the presence of impurities. After complete separation the ether should be removed by a pipette, and allowed to evaporate spontaneously from an open dish. The residue from the ether may be dissolved in distilled water, and again extracted with ether. To a drop of an aqueous solution of the ether residue apply the preliminary test with sulphuric and carbolic acid. To the remainder of the aqueous solution of the ether residue add an equal volume of a saturated solution of caustic potash, and evaporate the mixture on the water-bath. The double hydrate of potassium and diazobenzol will be formed if tyrotoxin be present, and this may be recognised by its properties and reactions which have already been described.