THE ANALYST.

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS.

ON THE PRESENCE AND DETECTION OF CYANOGEN IN JAVA, BURMA, AND HARICOT BEANS.

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(Read at the Meeting, May 2, 1906.)

As we had to deal with this question in the recent poisoning of cattle by "Java" beans, we have thought it might be of interest to put on record the result of our examination for cyanogen compounds of "Java" and other beans, as well as of peas of various kinds. As a fairly full report with regard to the poisoning cases in Scotland is given in the March and April numbers of the *Journal of the Board of Agriculture*, we shall not refer further to these, nor, indeed, to anything directly connected with them, but shall confine our attention to the various specimens which we procured for experiment.

For the determination of cyanogen we employed the method which Professor Dunstan used — namely, extracting the glucoside with alcohol, distilling off the latter, decomposing the former with hydrochloric acid, and distilling off the hydrocyanic acid (the *Agricultural Ledger*, No. 2, 1905). For the detection of the hydrocyanic acid we made a mash of about 10 grams of the ground beans with 20 or 30 c.c. of warm water in a small stoppered bottle and kept at 40° to 50° C. for half an hour, then applied a modification of the Schönbein test. This consisted in holding in the vapour in the bottle a piece of filter-paper, which had been first dipped in a 0.2 per cent. tincture of guaiacum resin, and then in a 0.1 per cent. solution of copper sulphate, when the paper assumed a fine blue colour almost immediately, if hydrocyanic acid was present. It is important that the blue colour should be rapidly developed, as the prepared paper slowly assumes a blue tint when exposed to the air, and this might be mistaken by careless observers for the effect of hydrocyanic acid. A good plan, therefore, is to prepare two slips of paper simultaneously, insert one into the vapour to be tested for hydrocyanic acid, and simply expose the other to the air, when a comparison will decide the question. If this plan is adopted no mistake can be made. In applying the Schönbein test it is generally directed to dip the filter-paper in a 10 per cent. tincture of guaiacum resin, and dry before dipping in the 0.1 per cent. solution of copper sulphate. On drying the paper, however, it

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always assumes a blue tint, and this vitiates its application to the detection of hydrocyanic acid. In consequence of this defect we have modified the test as described above.

It should be noted that, as a rule, hydrocyanic acid (if present) can be detected after half an hour's warning; but where no positive result is obtained, the bottle should be closed and allowed to stand at the ordinary temperature for several hours, in order to allow the hydrocyanic acid to accumulate by the gradual action of the enzyme contained in the bean on the cyanogenetic glucoside.

After deciding the method of procedure, we, in the first place, examined several specimens of Java beans, and found from 0·027 to 0·137 per cent. of hydrocyanic acid, this being, of course, understood to exist in the beans in the form of an amygdalin-like glucoside. These results practically agree with those of the uncultivated variety examined in the Scientific and Technical Department of the Imperial Institute. In order to determine whether there was really any difference as regards cyanogen contents in beans of different colour, we obtained an average sample, weighing about 3 pounds, of a lot of Java beans, and separated them into five different kinds. These consisted of white, brown of various shades, speckled, chiefly brown and white, with a few purple and white, purple of various shades, and black beans. The sample was composed of these as follows :

								Per Cent.
White beans	...	...	...	...	...	...	...	9
Brown beans	...	...	...	...	...	...	...	34
Speckled beans	...	...	...	...	...	...	...	33
Purple beans	...	...	...	...	...	...	...	20
Black beans	...	...	...	...	...	...	...	4
								<hr/>
								100

These were tested separately with the following results :

								Hydrocyanic Acid. Per Cent.
White beans	...	...	...	...	...	...	...	0·027
Brown beans	...	...	...	...	...	...	...	0·038
Speckled beans	...	...	...	...	...	...	...	0·038
Purple beans	...	...	...	...	...	...	...	0·031
Black beans	...	...	...	...	...	...	...	0·042
								<hr/>
Average	...	...	...	...	...	...	...	0·036

From these results alone it might appear that the white variety contained the least, and the black the most, hydrocyanic acid; but this distinction does not really hold good, although it has been held that white contains the least, and purple the most, of that ingredient. Other tests we have made showed in brown beans 0·072, and in black beans only 0·029 per cent. of hydrocyanic acid, so that the generalization as to the quality of the bean according to its colour is of no value whatever.

It has been stated that the cyanogen was contained in the husk and not in the kernel of the bean, but this opinion or assumption we have proved to be quite untenable. We carefully decorticated a small specimen of black beans, and found

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that the husk was equal to about 10 per cent. of the whole, while the percentage of hydrocyanic acid in the decorticated portion was 0·030 per cent., and that in the husk only 0·003 per cent. We also procured a large specimen of decorticated beans, and the corresponding husks, from the mixed varieties, and found in the decorticated portion 0·061, and in the husks 0·006 per cent. of hydrocyanic acid. It is thus apparent that the kernel contains at least ten times as much hydrocyanic acid as the husk, so that the statement we have referred to is not borne out by fact. It has also been suggested that it is the small, rounded beans that contain the hydrocyanic acid, but our experience does not bear this out even in the slightest degree.

Having dealt with the Java beans, we next procured specimens of various kinds of bean and pea, and tested them for hydrocyanic acid, with the following results, which include also the average weight of the beans :

	Hydrocyanic Acid. Per Cent.	Number of Beans in 100 Grams.
English beans	none	170
Morocco beans	none	—
Smyrna beans	none	100
Java beans	0·027 to 0·137	272
Rangoon or Burma beans	0·005	368
Large haricot beans	none	64
Large haricot beans	none	78
Small haricot beans (Rangoon)	0·009	384
Small haricot beans	0·006	420
Small haricot beans	0·001	448
Small haricot beans (Chili)	none	228
Paiyin beans	none	1960
Gram	none	—
Calcutta white and gray peas	none	—
Odessa and Mutter peas	none	—

The haricot beans (both small and large) mentioned above were obtained from different sources, and were all sold for human food. The sample containing 0·001 per cent. of hydrocyanic acid was purchased by ourselves in a grocer's shop in Glasgow.

From the above table we have further and most conclusive proof that it is a mistaken idea that the white bean is free from the cyanogen compound, as the latter was present in three specimens of the white haricot bean, which is almost certain to be a cultivated variety. It will be observed that the specimen of the ordinary (or coloured) Rangoon or Burma bean given above contains only rather more than half that contained in one specimen of the small white haricot beans.

In conclusion, we may note the effect of boiling the unground beans with water, and of mashing the ground beans with warm water. In carrying out the former experiments the three samples of small haricot beans containing hydrocyanic acid were steeped overnight with water and boiled until thoroughly cooked. They were then allowed to cool, beaten up with water, and the modification of Schönbein's test applied, but hydrocyanic acid could not be detected in any of them. The hydrocyanic acid was now determined in the beans originally containing 0·009 per cent., after this treatment, and 0·002 per cent. was found. These results show clearly that a large

proportion of the cyanogen compound and the whole of the enzyme have been destroyed by the boiling, but whether the amygdalin glucoside is harmless, seeing there is no enzyme present to produce hydrocyanic acid, is a question with which we cannot deal. In order to corroborate these results, Java beans containing 0.038 per cent. of hydrocyanic acid were steeped and boiled in the manner described; but after this treatment no hydrocyanic acid could be detected by the modification of the Schönbein test, although 0.019 per cent. in the form of the glucoside was actually present.

The result is entirely different when cold or warm water, as applied in the test referred to, is mixed with the ground bean. In this case we found that in two or three days practically the whole of the cyanogenetic compound was decomposed, and the cyanogen in it liberated in the form of hydrocyanic acid.

DISCUSSION.

Dr. VOELCKER remarked that Professor Dunstan drew a clear distinction between the white and the coloured beans, and spoke of the white beans as being cultivated and as containing no cyanogenetic glucoside, whereas the speckled and purple beans did contain one. He was inclined to think, however, that this was an assumption rather than the result of actual experiment, and accordingly the present paper, if the beans dealt with were botanically the same, would be of considerable value.

Mr. HENDRICK said that he had had occasion to examine a good many samples of these Java beans, and had done a little investigation on lines somewhat similar to part of that recorded in this paper. His results, generally speaking, were in accord with those of the authors. Every specimen that he had examined of these so-called Java beans, a great many thousands of tons of which had been imported into Europe during the last year or two, had yielded hydrocyanic acid in relatively large quantity. The beans varied very greatly in colour, even from bag to bag of the same consignment. In some cases white beans were more numerous than those of any other colour; in other cases the majority of the beans were black. He did not find, however, any corresponding variation in the amount of hydrocyanic acid, though, generally speaking, the darker beans contained a little more hydrocyanic acid than the light ones. He was not satisfied that the whole of the hydrocyanic acid which it was possible to produce from the beans was obtained by extracting the glucoside with alcohol, which was, he understood, the method used by the authors of this paper. In a few experiments which he had made he had found that mere soaking of the ground beans in water and subsequent distillation yielded, in every case that he had tried, a larger quantity of hydrocyanic acid than was obtained by the alcohol extraction method. He also had de-husked a sample of the beans, and had not obtained any hydrocyanic acid from the husks; it was all contained in the inner part of the beans. It had been stated that where the beans had been washed before being used there had been no poisoning. Through the kindness of certain large dealers he had been able to visit some mills where the beans were thus treated, and had seen the whole process. The washing process was a very brief one, and though it removed much dirt, it did not do more than clean the outside of the bean, and could not affect the amount of cyanogenetic glucoside present. His difficulty was to

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explain why there had not been far more cases of poisoning. Great quantities of the beans had gone into consumption. He personally knew of cases in which beans, of which samples from the same parcel yielded a large proportion of hydrocyanic acid, had been fed daily for weeks to cattle without ill-effect. In other cases poisoning had occurred at once, and large numbers of animals were lost. The poisoning seemed to be, so to speak, sporadic, and he felt convinced we had still something to learn as to its cause.

Mr. ESTCOURT said that he had been told by a miller near Manchester of certain beans which had poisoned a considerable number of cattle. These beans were described as "Indian mutter."

Mr. HENDRICK said that he had examined what were known as "mutter peas," and had not found them to yield any hydrocyanic acid. It was known, however, that these peas sometimes caused poisoning of a different kind (see a paper by Dr. J. A. Voelcker, ANALYST, xix., 102). In some cases the beans had been given in the form of dry meal, in some cases they had been soaked, and in some cases steamed. He had always found it quite easy to detect the hydrocyanic acid in these beans by smell, after soaking the meal in water for a short time.

Dr. LEATHER said that there was no doubt that these various beans, those from Burma especially, did contain a cyanogenetic glucoside, though it was not yet certain whether this was identical with the phaseolunatin of Professor Dunstan. It is uncertain, also, what the exact botanical nature of these beans is. They seem to be a mixture of species, but in India nearly all the crops are mixed. He thought, from what he had seen of these beans, that they probably all belong to the same genus, but it was not certain whether Professor Dunstan's statement as to *Phaseolus lunatus* is generally correct. Probably it is correct for the plant that Professor Dunstan operated on, but it is not certain that all these Burmese beans are the same. He had found a cyanogenetic glucoside in various Indian beans, and its presence seemed to be very common in leguminous plants in the East. The quantity of prussic acid obtainable varied considerably. It is very much to be desired, of course, that further knowledge should be obtained as to the physiological action of the glucoside itself. It is easy to understand the production of prussic acid by grinding up and soaking the beans in water, because these cyanogenetic glucosides are almost always associated with enzymes capable of hydrolysing them; but whether such hydrolysis would necessarily be brought about by the enzymes of the digestive organs is another question altogether, and, as far as he was aware, no work has been done in that direction. It seems very possible that the erratic effects of the beans might be explained by variations in the degree to which hydrolysis results from digestive action. In one of the first references made to him in relation to these Burmese beans, it was stated that the beans were consumed regularly by the prisoners in Burmese prisons. All that food was cooked, so that the enzyme in it was certainly destroyed; and it remained then for the enzymes of the digestive juices to hydrolyse the glucoside if they could. Apparently, as a rule, they could not. In the case of stock-feeding it is clearly necessary that such beans shall first be cooked, so as to destroy the enzyme; but it is to be recollected that the glucoside is not destroyed by a temperature of 100° C.

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Mr. HENDRICK asked whether Dr. Leather knew of any case of poisoning from Burma beans.

Dr. LEATHER said that he only knew of cases of poisoning that had occurred in this country; none had occurred in India.

Mr. HENDRICK said that, as far as he knew, there had been no case of poisoning by Burma beans. It was at first stated in the newspapers that some of the beans which had caused poisoning were from Burma, but in every case, so far as he was aware, it was found on investigation that they were Java beans. The latter were different from Burma beans. Both seemed to be varieties of *Phaseolus*, though he did not know the exact species. The Burma beans were quite uniform.

Dr. LEATHER: No, they vary very much indeed.

Mr. HENDRICK said that all the samples which he had seen of what was described as "Red Burma Beans" were red mottled beans, very similar to one another in appearance, whereas the Java beans he had seen varied greatly in colour and appearance. These red Burma beans have been imported in large quantities for many years past, and are extensively used as cattle-food in this country, but he had never heard of an authenticated case of poisoning arising from their use.

NOTE TO DISCUSSION BY THE AUTHORS.—Our experience differs from that of Mr. Hendrick with respect to the extraction of the glucoside with alcohol, as this method gave more cyanogen than that by simply soaking the ground beans with water and distilling.