ON THE PHYSIOLOGICAL ACTION OF THE CALABAR BEAN (PHYSOSTIGMA VENENOSUM, BALF.)

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XLVIII.—*On the Physiological Action of the Calabar Bean* (Physostigma venenosum, *Balf.*). By THOMAS R. FRASER, M.D., Assistant to the Professor of Materia Medica in the University of Edinburgh. Communicated by Professor CHRISTISON, M.D., D.C.L., V.P.R.S.E.

(Read 17th December 1866.)

In 1855, the Professor of Materia Medica in the University of Edinburgh, in a paper read before this Society, directed the attention of physiologists to some of the remarkable properties of the Calabar bean.* In 1862, I presented a graduation thesis to the University of Edinburgh on the "Characters, Actions and Therapeutic Uses of the Ordeal Bean of Calabar." The principal results I had obtained at that time were that this substance causes death by either syncope or asphyxia, the latter being due to an effect on the spinal cord and on the respiratory centres; that the symptoms resemble those of cardiac or pulmonary embarrassment, according to the quantity of the poison administered, and to its rate of absorption; and, also, that the topical application of this agent to the eyeball, or to its neighbourhood, produces a marked and rapid contraction of the pupil and various disturbances of vision.† Since then, and more especially because of the peculiarity of the last of these conclusions, a lively interest has been taken in this substance. Its actions on the eye have been investigated by nearly all the leading ophthalmologists of Europe and of America, and its general physiology has occupied the attention of many distinguished students of biology. Nor have these labours been barren of practical results. Ophthalmic medicine has adopted this agent as one of its important remedies, and there can be little doubt that general medical practice will soon include in its Pharmacopœia a drug of so great energy.‡

The present investigation was undertaken for the purpose of extending and supporting my previous results, with some of which subsequent observers have disagreed; but I purpose to take an opportunity of examining these discrepancies with some detail in a different place. The effects which follow the topical application to the eyeball will be merely alluded to in this paper, as this portion of the subject has not been completed. Enough has, however, been done to convince

* Proceedings of the Royal Society of Edinburgh, vol. iii. p. 280; and Monthly Medical Journal, vol. xx., 1855.

† Edinburgh Medical Journal, 1863, and pamphlet.

‡ Since this sentence was written the *Physostigmatis Faba* has been admitted into the edition of the "British Pharmacopœia," published in 1867.

me of the insufficiency of the views hitherto advanced, and to suggest the advisability of extending my observations.

Preparations.

In 1863, I separated from the kernel, from the spermoderm of the bean, and, also, soon after, from the excrement of a lepidopterous insect which feeds on the kernel,* an amorphous active principle, possessing the general properties of a vegetable alkaloid, for which I proposed the name Eserinia, derived from Eseré, the usual name of this ordeal-poison at Calabar; and with it a few experiments were made, some of which have been published. Shortly afterwards, I succeeded in obtaining this alkaloid in, apparently, a state of greater purity, and as a crystalline substance, to which I gave the name Eseria. A crystalline acid, having a similarity to, and being probably identical with, tartaric acid, was also obtained from the kernel at that time. In the present investigation, however, an extract, prepared by acting on the finely pulverised kernel with boiling alcohol (85 per cent.), has been used. This preparation contains a considerable proportion of fatty matter, which prevents its complete solution in water; and, as the division into separate doses of a mere watery suspension would lead to many inaccuracies, it was found necessary to weigh the requisite quantity, separately, for the majority of the experiments. This extract is hygroscopic, which further required that it should be dried and kept in an exsiccator in order to ensure an unvarying preparation.†

Subjects of Experiment and Comparative Effects of Doses.

With few exceptions, the experiments were made with the common frog (*Rana temporaria*), birds, and various mammals. It was found that fatal results were produced with the smallest quantity on birds; and that the largest doses, in proportion to weight, were required by amphibia. A dose of one-sixteenth of a grain proved rapidly fatal to a pigeon weighing nine ounces and three-quarters; whereas a frog, which weighed 726 grains, has recovered from three grains of extract—a quantity sufficient to produce death in a dog of average size.

A. ACTION THROUGH THE BLOOD.

As I have already, in a previous paper, described with considerable detail, the general symptoms which follow the administration of physostigma, it will be unnecessary to give them here. It has also been shown, on the same occasion, that the more rapid the absorption of the poison the more quickly are fatal effects produced, and that the active principle may be absorbed by any living

* On the Moth of the Esere, or Ordeal Bean of Old Calabar. The Annals and Magazine of Natural History, May 1864, pp. 389–393.

† The varying potency of an extract possessing the property of absorbing moisture may unfit it for therapeutic purposes, but the tincture I have already recommended (*op. cit.* sect. iii.) will prove a sufficient substitute, and it has the great advantage of constancy of strength.

tissue. From the following experiment it is proved that prolonged digestion with gastric juice does not impair the energy of Calabar bean:—

Experiment I.

A gastric fistula was formed in a healthy dog, and, some days afterwards, and while the animal was in good health, 500 grains of gastric juice were withdrawn from the stomach. Four hundred grains of this were mixed with half a grain of extract of physostigma, received in a flask with an arrangement to impede evaporation, and placed in a water-oven at a temperature of 98° F. The digestion was continued for twenty-four hours, when the fluid was placed in a capsule and evaporated at 85° F. The resulting extract was finely pulverised, heated with alcohol of 85 per cent., filtered, and again evaporated to dryness. Contact with distilled water removed an acid fluid, which was made alkaline by excess of magnesia, and agitated in a bottle with chloroform. The chloroformic solution was removed by a separating funnel and evaporated, and the resulting brown extract was suspended in distilled water. A drop of this was applied to the conjunctiva over the right eyeball of a rabbit, whose pupil, before the experiment, measured $\frac{1}{8}\frac{1}{8}$ths × $\frac{1}{8}\frac{1}{8}$ths of an inch. In eight minutes, the pupil was $\frac{1}{2}\frac{5}{0}$ths × $\frac{1}{8}\frac{1}{8}$ths; in fifteen minutes, $\frac{1}{3}\frac{5}{0}$ths × $\frac{1}{8}\frac{1}{8}$ths; in twenty minutes, $\frac{1}{4}\frac{5}{0}$ths × $\frac{1}{2}\frac{5}{0}$ths, and it continued in this contracted condition for many hours. The remainder of the fluid was injected under the skin of a young pigeon, and caused its death in eight minutes.

Several small pieces of hard-boiled white of egg were placed in a flask with the remaining 100 grains of gastric juice, and digested under exactly the same conditions, and at the same time, as the extract of physostigma. They were found to be completely dissolved in less than ten hours. There could, therefore, be no doubt as to the activity of the gastric juice which had been employed.

This merely confirms the result before obtained, of fatal effects following the introduction of the poison by the digestive system.

I believe that BRINTON first demonstrated that a poison which had been administered by the blood may be excreted by the stomach and intestines.* This was proved with tartar emetic; and, more recently, TAYLOR has published evidence showing that arsenic also may appear in the stomach although it had not been administered by the alimentary canal.† I took an opportunity of examining if a similar event occurs in poisoning with physostigma.

Experiment II.

Five grains of extract, suspended in water, were injected into the right jugular vein of a dog, and caused the death of the animal in eleven minutes. The stomach was immediately removed, and its contents, along with some of its mucous coat, obtained by scraping, were partially dried at a low temperature, and then boiled with successive portions of spirit (85 per cent.) acidulated with tartaric acid. The tincture was concentrated by distillation, and then evaporated to dryness. The extract was treated with distilled water, filtered, and agitated with ether until the fatty matters were removed. The remaining watery solution was made alkaline by the addition of carbonate of sodium and shaken with ether; and the ethereal solution was distilled. A yellowish, alkaline, amorphous residue was obtained, weighing three-fifths of a grain, and having a disagreeable animal odour. A minute portion of this extract was mixed with two

* Cyclopædia of Anatomy, article "Stomach;" Lancet, 1853, vol. ii. p. 599; and Lectures on the Diseases of the Stomach, 2d edit., 1864, p. 54.

† Guy's Hospital Reports, vol. vi. p. 397.

drops of distilled water and applied to the conjunctiva of a white rabbit, in the presence of my friend, Dr Crum Brown. Before the application, both pupils had a diameter of $\frac{1}{4}\frac{1}{8}$ths of an inch, in a full light. At first a little irritation was caused. In thirteen minutes, the pupil had contracted to $\frac{1}{4}\frac{1}{8}$ths, and in eighteen minutes to $\frac{1}{8}\frac{1}{8}$ths; the other pupil still remaining at its original diameter of $\frac{1}{4}\frac{1}{8}$ths. This extreme contraction continued for upwards of an hour; but in two hours the pupil was $\frac{1}{6}\frac{1}{8}$ths, and by the following morning it had resumed its original diameter.

It would, therefore, appear that physostigma, when administered by a vein, finds its way into the stomach—a method of poison-excretion which has been established in the cases of antimony and arsenic.

Although this investigation has for its principal aim the determination of the exact method in which physostigma acts, and the demonstration, as far as possible, of the histological structures which it influences, it may be necessary to describe, at this place, the general symptoms which follow the administration of a poisonous dose. In the case of mammals, I have already entered fully into this subject in a previous paper, from which I extract the following descriptions:*—

"When a *small* fatal dose is administered to one of the lower animals, a train of symptoms is produced usually in the following order:—A slight tremor is first seen, especially at the posterior regions, and this extends forwards to the anterior extremities and the head. The limbs yield immediately afterwards, the posterior becoming generally first paralysed, and the animal lies extended in a state of almost complete muscular flaccidity. A few attempts may be made to recover the normal position, but they are usually ineffectual. The bowels, in most cases, are evacuated, and urine is passed. The pupils generally *contract;* as the symptoms advance, the respiration becomes slow and irregular, with a distinct stertor accompanying both inspiration and expiration, and frothy mucus escapes from the mouth. Muscular twitches occur, and often continue after respiration has ceased. Reflex action cannot be produced by either pinching or pricking the skin. By-and-by the eyelids do not contract when touched or even when the eyeball is pricked. On lifting by the ears, the limbs hang inertly, and the only sign of life is an occasional gasping inspiration, which also soon ceases, and the animal appears dead.

"Consciousness is preserved during the whole time, until the power of expression is lost. During incomplete paralysis, proofs of sensation may be obtained by pinching the ears or pricking the skin. Immediately after death the pupils dilate.

"On opening the body the various muscles which are cut contract. The diaphragm and muscles of the extremities may be excited to action by pinching the phrenic and sciatic nerves, and the contractility of the muscles generally is retained for some time after death. The heart is found acting regularly, and the intestines exhibit distinct vermicular action. The heart may continue its action

* *Op. cit.* sect. ii.

for one hour and a-half after death. Its chambers usually cease to contract in a definite order, the left auricle first losing its spontaneous action, then the right and left ventricles, and, after an interval, the right auricle. The large veins in the thorax are found distended. . . . The lungs are engorged—in two experiments this had proceeded to such an extent that detached portions sank in water. . . .

"When a *large* fatal dose of the kernel is administered, the hind limbs almost immediately yield, and the animal falls. It lies flaccid, and in any position, on the table, and exhibits muscular power only by a few twitches. The pupils contract; in a few cases fluid escapes from the nostrils and mouth, and the lachrymal secretion is increased. Reflex action cannot be produced by irritation, and the respirations, after a few gasps, cease.

"The pupils dilate immediately after death. On opening the body, muscular twitches occur. . . . The heart is found distended and passive; irritation, however, produces contraction for about ten minutes after death. The vermicular action of the intestines is very much diminished, and can scarcely be observed. . . . The mesenteric arteries and veins may be readily distinguished by the colours of their contents."

The following will serve to illustrate the symptoms with frogs:—

Experiment III.

Three grains of extract of physostigma, suspended in twenty minims of distilled water, were injected, by Wood's syringe, into the subcutaneous cellular tissue at the back of a light-coloured frog, weighing 430 grains. For four minutes it appeared perfectly unaffected and jumped about normally; after which time some increase occurred in the respiratory irregularity which is always found in frogs. In seven minutes, the respiratory movements of the chest had ceased; but those of the throat continued for other four minutes (eleven after injection). About this time, the movements of the animal were sluggish; the fore legs gradually began to separate until they no longer supported the chest and head; and the posterior extremities were affected in a like manner, and soon after lay extended and flaccid. Weak voluntary movements, however, continued until fifteen minutes after the exhibition of the poison; and, for some time after this, irritation demonstrated the continuance of reflex power. In half an hour, the skin of the frog had undergone a marked change, having become of a dark brown colour. Although now apparently dead, it was not, in a strict physiological sense, really so. Motor nerve-conductivity was retained for many minutes longer; the diastaltic function was not abolished, and, hence, it was possible to show that afferent nerve-conductivity also continued; and the muscular tissue, for many hours, contracted when stimulated, and, in the case of some of the heart chambers, spontaneously and successively did so, for a shorter period.

Into all these, and many other points, it is necessary to enter with detail, and several of them may be overtaken in a somewhat connected manner by examining the cause of what is the most prominent, as well as one of the earliest, of the phenomena described. This is obviously the condition of gradually increasing paralysis.

Action on the Voluntary Muscles.

The peculiar successive tremors, which are observed in warm-blooded animals, at first sight suggest that the paralysis caused by Calabar bean is due to an affection of the muscular system; and the condition of general flaccidity, which so rapidly follows its administration to frogs, appears to favour, as it certainly does not contradict, this opinion. Without pretending that such was the order followed in this investigation, it will, as a matter of convenience, be advisable to examine, in the first place, the effects which are produced on voluntary muscles.

Experiment IV.

A full-grown active rabbit had injected into the subcutaneous tissue of its right flank, three grains of extract, suspended in eleven minims of distilled water. Tremors occurred in two minutes; the anterior extremities soon after yielded; and, in four minutes and thirty seconds, the animal fell, the muscular trembling having increased in vigour and having become general over the body. Respiration ceased in five minutes after the injection, but muscular tremors continued during other three minutes. When the thorax was opened the heart was found dilated and passive. Twenty-four minutes after the administration, galvanic stimulation of the sciatic nerves caused powerful muscular contractions; within thirty-six minutes, these nerves were completely paralysed, though application of the electrodes to any of the voluntary muscles produced marked contractions. These contractions became gradually weaker, but could be distinctly excited until one hour and thirteen minutes after the poison had been exhibited.

The general result in all the other experiments which were performed on warm-blooded animals was the same. Muscular contractility remained after destruction of the function of motor nerves; and this also occurred, in even a more marked manner, with frogs.

Experiment V.—*(Temperature of Laboratory about 53° F.)*

By means of Wood's syringe, I injected three grains of extract, in fifteen minims of distilled water, into the lower portion of the abdominal cavity of a frog which weighed 473 grains. The usual phenomena quickly occurred. In sixteen minutes, the sciatic nerve and the neighbouring muscles of the left thigh were exposed and found active.

The muscles were now of a very blue colour, quite distinguishable from their normal appearance; and this colour change was discovered in the serous and fibro-serous tissues also. In about four hours, motor nerve-conductivity was universally destroyed. The heart contracted rhythmically, and at a very reduced rate, until twenty-six hours after the administration, after which, the auricles contracted more frequently than the ventricles, and continued to do so until the heart's action ceased, seventy-three hours after the poison was injected; and, by microscopic examination of the web, it was found that a more or less feeble circulation was all this time maintained. Until this stage, no apparent change occurred in the readiness and vigour with which the striped muscles contracted when directly galvanised; their reaction continued to be alkaline, and they were perfectly flaccid. Soon after the stoppage of the heart's action the blue colour, which has been already mentioned, began to disappear, and in ninety-six hours (four days) the muscles were quite pale. No stiffness was yet observable, and galvanism still induced faint contractions. Rigor mortis commenced soon after this, but its progress was extremely slow, as galvanism produced dimples at the electrodes until 110 hours. When the frog was again examined, at 129 hours after the injection of physostigma, no muscular contraction could be produced by powerful galvanism; rigor mortis was complete; and the reaction of the muscles was found to be acid. Galvanism could produce a very faint contraction of the cardiac muscle,

limited to the points of stimulation, until about the time up to which feeble indications of retained contractility could be obtained in the voluntary muscles.

Experiment VI.—(*Temperature of Laboratory between* 52° *and* 54° *F.*)

To a frog, weighing 379 grains, four grains of extract were administered in the same way as in the preceding experiment. Motor nerve-conductivity ceased in two hours and sixteen minutes, by which time the exposed muscles were found to have become blue. The cardiac action continued rhythmical, though much reduced in frequency, until twenty-seven hours and fifteen minutes, after which, the auricles alone contracted spontaneously till forty-four hours, and then, spontaneous cardiac action entirely ceased. During all this time, the muscles were flaccid, contracted vigorously on the application of weak galvanism, and had an alkaline reaction and a blue colour. Soon afterwards, they became paler and slightly stiff, but it was not until seventy hours after the administration of the poison that galvanic stimulation failed to produce any contraction; and then rigor mortis, with an acid reaction of the muscles, set in.

These three experiments distinctly prove the absence of any paralysing effect by physostigma acting through the blood on striped muscle.

Rigor mortis is delayed for an unusual period after apparent death in cold-blooded animals, and its appearance, in mammals and birds, is certainly not hastened. In both classes this change in the condition of muscles is only indirectly affected by this substance, and that through its influence on the cardiac contractions. When the blood supply of the muscles is stopped their function is suspended, and rigidity follows; but the resulting rigor does not seem to be due, in any other than this indirect method, to the action of physostigma.

This may be more clearly demonstrated by detailing one of many experiments in which a portion of the frog was protected from the influence of the poison.

Experiment VII.—(*Temperature of Laboratory between* 52° *and* 54° *F.*)

The right iliac artery was exposed, by removing a portion of the sacrum, and tied in a frog, weighing 878 grains. Two minutes afterwards, three grains of extract, suspended in ten minims of distilled water, were injected into the subcutaneous cellular tissue at the right shoulder. In a few minutes, a condition of general paralysis existed, and shortly afterwards the skin of the tied limb was much paler than that elsewhere, this contrast becoming more marked as the experiment advanced. In an hour and twenty minutes, the sciatic nerves being exposed, it was found that the left was completely paralysed; while galvanism, applied to the right nerve, or that of the limb protected from the action of physostigma, produced active muscular contractions. The muscles of the tied limb were pale as contrasted with those to which the poison had access, and the latter were distinctly blue in colour. The non-poisoned muscles continued active until forty hours; but when examined at forty-nine hours they were acid and stiff, and did not contract when galvanised. In the poisoned parts, the functions of the motor nerves were destroyed in three hours and ten minutes; the non-poisoned, or right, sciatic continued active until thirty-two hours. It was possible to distinguish the heart's impulse on the thoracic walls, and to determine the frequency of its contractions. During the three days that immediately followed the poisoning, these steadily continued at a rate varying from seventeen to twenty-one; and on exposure, at the end of that time, fifteen feeble beats per minute were occurring. Soon after, the usual irregularities were observed, but the circulation was maintained until eighty-two hours after the injection of physostigma, as the microscope demonstrated. During all this period, the muscles everywhere, except in the tied limb, were flaccid, blue and of alkaline reac-