Thesis

Cut on narrow band to holl. Thos. Le Water 1881

Cape of Good Hope
Thesis

Address:

Ernst Reint
Cape of Good Hope

Thos. L. F. Bates
Dec. 18
Apocynin a Cardiac Poison
in Radix Apocyni Cannabis.
The Radix Apocyni mentioned by
Hussmann is in a class with several
other substances as very probably
containing active principles of the
nature of glucosides, has on in-
vestigation indeed shown itself to
be exceedingly active. This activity
however is not due as is supposed
to the presence of a glucoside.
The Root is obtained in Commend
in pieces ranging in length from
2 to 6 or 8 inches and in thickness
up to 3/4 of an inch diametrically
although generally not more than 3/4.
When dry it is very brittle but in
the fresh state tough and tenacious.
The roots spread at right angles
their junctions being marked
by large nodes.
It consists of a wrinkled, or longitudinally grooved cortex which is thin and tends to crack circularly at intervals like that of Spearmana, is yellowish-brown in colour and easily separable from a faintly yellow-coloured and somewhat porous pith. Both are extremely and persistently bitter, the perception of the bitter taste being preceded by slight itch. The powder is slightly irritating to the olfactory mucous membrane, acting as a mild stimulant, not nearly as powerful as produced Cassy Barks. To determine whether it had poisonous properties and if what general notion these were an extract was prepared from a small quantity of the root and injected subcutaneously in a frog; the injection took effect very quickly for on opening the thoracic cavity shortly after the heart had ceased beating.
For the purpose of ascertaining now to what constituent the active properties were to be ascribed about \( \frac{3}{4} \) of a kilogramme of the root was taken and ground into a tolerably fine powder, this was repeatedly extracted with absolute alcohol; all the extracts were then mixed, left perfectly quiet till quite clear, and now poured off. Distillation of the alcohol was now effected, and the retort washed out with boiling distilled water. The resulting solution was treated with oxide of lead and filtered, while hot; after continued washing with boiling water, alcohol was used to dissolve any of the active principle remaining in the precipitate. The filtrate was evaporated to half the quantity and when cold well shaken with sulphuric ether, the ether decanted and the process repeated 4 or 5 times.
On distillation of the ether a thick brownish fluid was left, this solidifying into an imperfectly crystalline mass. Small quantities of distilled water were now added, which dissolved the crystalline part, leaving a brown amorphous substance undissolved. On evaporation of the solution innumerable small crystals were deposited, which after purifying were coloured; they are soluble in cold, more readily in hot water also in alcohol and ether. Treated by boiling with Sulphuric Acid no change was produced; the addition of Sulphate of Copper in Solution 2 to 3 Pounds and then boiling were likewise without effect, indicating thus that it was no glucoside. Some of the crystals were now boiled and concentrated Sulphuric Acid added, a yellow colouration was
produced as in the case of Digitaline but no further change was observed on adding some crystals of Prussic Acid or Phosph as in the latter. Solution of the substance injected under the skin of a frog produced such slight modification in the heart's action that it might be attributable to imperfect purification rather than any essential properties of its own. The brownish putty-like substance after separating the crinoid string substance remaining was very sticky and tenacious & exhibited great activity. A solution of an opalescent character was formed by the addition of large quantities of boiling water but however remaining insoluble, the solution was filtered and shaken up with Carlo Animalia Pacifica. The bitter principle was absorbed by the charcoal which was collected on
a filter & treated with absolute alcohol this dissolved out the active bitter substance. After evaporation of the alcohol and repetition of the whole process for further purification a substance was obtained which may be called Apocynin. Its characters physical, chemical & physiological.

The alcoholic solution crystallized partly into minute needles sometimes arranged in a stellar manner partly it solidified into whitish polygonal masses. It is very sparingly soluble in cold water but on heating the water it melts into an amber-coloured resinous looking substance and is then dissolved, the solution becoming faintly opalescent. In alcohol & ether it is readily soluble, the solution in these instances remaining quite clear.

Apocynin boiled with Hydrochloric Acid & then with Cupric Sulphate acid
and Poole underwent no change; the application of the ferrocyanide of potassium test for determining the presence of nitrogen likewise gave a negative result; thus it is neither a glucoside nor on the other hand a nitrogenuous substance. In chemical characters it therefore bears a close resemblance to Digitoxin.

Regarding its physiological action the following experiments were made:

I. On frog (R. temporaria)
   a) large frog heart exposed
   11.42 a.m. 1/4 milligramme of Apocynin
   in solution injected intra-arterially
   11.45 irregular contraction of ventricle of peristaltic character, auricles hyper-extended.
   11.47 Ventricular contraction more regular & powerful
11.50 return of irregular peristaltic ventricular contractions; foamy
struggles, respiration deep and labored
11.52 Ventricles is no longer distended
by the contracting auricles, blood
only admitted into a small
pouch formed by the ventricle, the
rest firmly contracted.
11.52.30 Ventricular substance strongly
contracted, anemic appearance.
Auricles immensely distended.
The auricles continued to attempt
to expel the blood from them for
about 15 minutes longer.

(c) Middle SIZED Frog
at 12.10 p.m injection subcutaneous
of ½ m.g.
12.13 peristaltic contraction of
Ventricle
12.14.30 complete paralysis of ventricle
12.20 heart ceased beating.
(c) A weaker solution was now prepared and at 12.39 p.m. injection of \( \frac{1}{2} \) m.g. in a middle-sized frog.

12.45 Some slight irregular contractions of ventricle.

12.50 Apex of heart contracting more firmly, indicated by its greater whiteness in cuspule of the vent.

1. Heart still contracts well.

1.15 Condition unchanged.

3.10 Heart beating apparently quite normally, some weakening in contr. power.

(1) Heart exposed in frog, weight 35 grammes.

3.20 p.m. Injection under skin of \( \frac{1}{4} \) m.g. Apocyprin.

3.25 Irregular ventricular contractions begin.

3.30 Contraction somewhat peculiar (continues)
(d) 4 p.m. part of ventricle remains undistended in diastole; deep inspiration of frog at intervals.
4.30 same condition
5. no further change

(e) Frog weight 27 grammes
4.32 injection of 1/4 m. gr.
4.36 peristaltic contraction of ventricle, great distension of auricles; struggling of frog
4.40 ventricle stops beating, firmly contracted

(f) Frog weight 34 grammes
5.30 p.m. injected 1 m. gr.
5.38 first appearance of irregularity in ventricle contraction
5.45 heat stopped for a moment
hyper-distended but on struggling of frog recommenced beating
6. lower half of ventricle contract, now firmly, does not distend further.
6.3 apex of heart remains contracted no blood reaching it on contraction of auricles, rest of ventricle inclined to pulsate.

6.4 ventricle firmly contracted auricles paralyzed from our dictum.

2) Frog weight 33 grammes
3.43 p.m. injected 1/4 m. gr.
3.53 paralysis; firm contract of ventricle.

3) Frog small
injected rather more than 1/8 m. gr.
at 4.18; no marked effect; product heat still beating on the following morning.

From these experiments it appears then that the minimal quantity producing paralysis of the heart is
Poison is about 1/4 of a milligramme. In no case was it produced by an 1/20 m. gr. and in one experiment with a large vigorous frog 1/4 m. gr. which proved sufficient in all the other cases, was found too small a quantity to completely paralyse. The time which elapsed after the injection of the opium before the ventricle was brought to a standstill varied from a few minutes to more than half an hour depending upon the size of the frog. Peristaltic contraction of the ventricle was produced alike by the smaller or larger quantities but in the case of the smaller the heart recovered itself after some time. In some cases the auricles likewise appeared to be affected by the poison part of their walls contracting firmly and becoming distended by the blood
Direct application of the solution to the heart was found to be attended with the same result as the subcutaneous injection.

Experiment on Dog with Apocynin for determining alteration of Blood Pressure

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<th>Pulse (per minute)</th>
<th>Remarks</th>
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<td>12.15</td>
<td>94</td>
<td>15</td>
<td>Inj of 1mg 50% Apocynin in Stein into jugular vein</td>
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Height of Dog = 5900 grams
Dog enanized & artificial respiration
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<td>12.32.10</td>
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<td>Steppage of head</td>
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Beginning irregular in heart's contraction
Irregular at times, strong pulsations
In this experiment on the dog thus far as soon as the camphor had produced its full effect and the heart was beating regularly 1 mg. was injected into the jugular vein in which a small cannula had been fixed. The heart soon showed signs of the activity of the substance; the blood pressure rose steadily and reached its maximum within 9 minutes, then slowly diminished with at intervals very markedly irregular contractions of the heart well indicated by the tracing. 19 minutes after injection the pressure fell to zero and the heart had stopped beating. On opening the thorax as was to be expected, the ventricles were sharply contracted and the auricles distended with blood. Experiments on Cat 2
Rabbit likewise gave results which although not as markedly characteristic of requiring injection of a larger quantity were confirmatory of that on the Dog showing that Apocynin is an active cardiac poison bearing a close relation to Digitalis, producing more powerful contraction of the heart, great increase of the blood pressure & ultimately stoppage of the heart's systoles of the Ventricles.

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M.B., O.M.