The physiological action of the oil and seeds of Croton elliotianus from British East Africa / by J. Theodore Cash and Walter J. Dilling.

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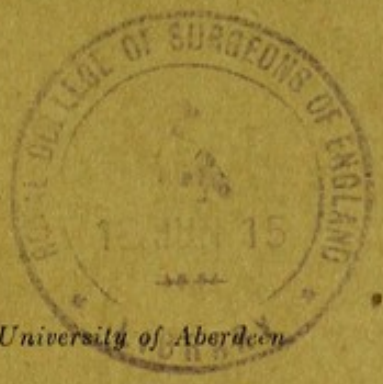

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THE PHYSIOLOGICAL ACTION OF THE OIL AND SEEDS OF CROTON ELLIOTIANUS FROM BRITISH EAST AFRICA

J. THEODORE CASH AND WALTER J. DILLING

From the Pharmacological Laboratory, University of Aberdeen

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Some time ago we received from the Imperial Institute seeds of a Euphorbiaceous plant (Croton Elliotianus) together with a sample of their contained oil. The nature of this oil had been under chemical investigation at the Imperial Institute and Professor Dunstan suggested that an examination of its physiological action would prove of interest. The seeds in question are derived from a thinly branched tree 10 to 15 meters high, growing in the vicinity of Nairobi, British East Africa, at an altitude of 1800 to 1900 meters. The dry lamina is 8 cms. in length by 3 broad, the upper surface being of a dull dark brown color whilst the under surface presents a thick scaly covering. The petiole is 4 to 5 cms. in length. The inflorescence, 20 cms. in length, is openly arranged, the individual flowers being 1 cm. long and stalked. Capsule nearly 3 cms. long by 2.5 broad, oblong, slightly tricarinate, three-seeded. Seeds smooth, ecaruncu-late.[1]

(After a brief description of the seeds of Croton Elliotianus has been given it is proposed to discuss in this paper their pharmacological action with reference to man and certain of the lower animals. The action of the oil-free seed and of the oil itself will also be considered.)

[1] Bot. Jahrbüch f. syst. Pflanzengesch. u. Pflanzengeographie. 1904, v. xxxiii, p. 289. C. F. Elliot, Comm. A. Sufler, 31, x, 1902.

DESCRIPTION OF THE SEED

Viewed from above the seed is oval, slightly beaked at its anterior and truncated at its posterior extremity, convex upon the dorsum, which has a faintly marked median ridge; the ventral surface shows two flattened facets separated by a longitudinal ridge which is approximately median in position. The testa, which is thin but resistant, is of a dull chestnut to reddish-brown tinge, its surface is convoluted owing to the presence of many

FIG. 1. SEEDS OF CROTON ELLIOTIANUS ENLARGED (⅔)

a, dorsal surface; *b*, ventral surface of seed intact; *c*, endosperm showing part of tegmen and testa in situ. The centimeter scale is correspondingly enlarged.

primary and secondary ridges which are separated by shallow sulci, the appearance being roughly suggestive of the cerebral surface.

The tegmen, which on breaking the testa remains attached to the fragments, is delicate and silvery. The endosperm fully occupies the cavity formed by the seed coats, it is waxy and buffish-yellow in appearance, evidently oil-containing, and mark-

ed with convolutions in correspondence with those observed upon the testa. The embryo is upright and has broad cotyledons; the albumen is abundant.

Measurement (a) and weight (b) of seeds

a. Eight seeds were measured, the individual results yielding the following averages: greatest length, 17.625 mm.; width,10.53 mm.; thickness, 6.9 mm.

b. Average weight of the same eight seeds, 0.6925 gram; average weight of the seed coats, 0.32675 gram; average weight of the endosperm, 0.36575 gram.

Variation between individual seeds in size and weight is not extensive; out of thirty-three seeds the greatest difference between any two in length was 1.75 mm., in width 1.4 mm., in thickness 0.6 mm., and in weight (seed intact) 0.245 gram. The odor of the seed is faint but peculiar, identical with that of the contained oil, the taste is slightly unpleasant. Subsequent to chewing a sensation of dryness with discomfort develops, especially upon the soft palate. Triturated with potassium hydrate solution the seed content is saponified or, rubbed with mucilage, a white emulsion is formed.

The oil of Croton Elliotianus seed

Investigations at the Imperial Institute have shown that the seeds contain 27.7 per cent of a yellowish oil, consisting principally of the glycerides of unsaturated acids (about 90 per cent) and of saturated acids (about 10 per cent). The unsaturated acids are chiefly linoleic acid and small quantities of oleic acid, whilst the saturated acids consist chiefly of palmitic acid. Indications were also obtained of the presence of hydroxylated fatty acids. The oil of Croton Elliotianus does not contain the acrid vesicating resins which occur in the oil of Croton Tiglium (Dunstan and Boole).[2] The analytical constants furnished by two samples of Croton Elliotianus Oil are compared in the following table with those given by the oil of Croton Tiglium:

[2] Dunstan and Boole: Proc.Royal Society, London, 1895, vol. lviii, pp. 238–240.

	OIL OF CROTON ELLIOTIANUS		OIL OF CROTON TIGLIUM
	Sample 1	Sample 2	
Specific gravity at 15°C.	0.9266	0.927	0.9428
Acid value*	4.24	3.6	
Saponification value*	201.5	191.6	201–215
Helmer value (percentage of insoluble acids)	94.0	94.8	88.9–89.1
Iodine value, per cent	138.5	147.0	101.7–102
Titer test (solidifying point of fatty acids)	13.7–13.8°C.	about 140°C	

* Milligrams of potash required for one gram of oil.

ACTION OF INGESTED SEED UPON MAN

These observations were made on a healthy adult, leading a uniform life, receiving a mixed but simple dietary and having one alvine evacuation with perfect regularity once in twenty-four hours. No alcohol was taken during the observations. The symptoms were recorded as they arose. A short epitome of results following ingestion of progressive doses of the seed is here inserted.

A fresh seed was deprived of its coverings, the desired quantity of it weighed, the fragment thoroughly chewed and swallowed with 20 cc. of water. Thereafter a like amount of water was employed in rinsing the mouth and was then swallowed. The observations were commenced as nearly as possible midway between breakfast and lunch or lunch and dinner.

0.075 gram. This weight of seed caused a slight and passing sensation of roughness of the posterior part of the soft palate. No discomfort nor active purgation ensued but there was evident diuresis whilst the dejection on the following morning (fifteen hours) was unwontedly soft in consistency. (This was the smallest dose causing a noticeable effect in the case of this observer.)

0.1 gram. Effect upon the palate persisted longer, borborygmi two hours after ingestion. Diuresis. (The increase in urine for two distinct observations in which this dose was taken was found to be 220 cc. and 285 cc. respectively for the twenty-four hours succeeding ingestion (the dietary being identical), dejecta on the following morning soft though formed.)

0.2 gram. Followed in twelve minutes by sensation of roughness and dryness on soft palate.

130 minutes. Active borborygmi, no discomfort but slight gastric nauseation.

145 minutes. Distinct tendency to evacuation.

293 minutes. One copious fluid dejection unattended by griping, succeeding night once disturbed by recurrent tendency but this was relieved by copious diuresis.

14 hours 45 minutes. Morning dejection soft and only partly formed. No further effect.

0.4 gram. Local effect on soft palate and posterior dorsal region of tongue experienced in ten minutes, became distinctly unpleasant.

60 minutes. Clear mucus separated from pharynx, distinct errhine effect, slight gastric discomfort and nausea.

80 minutes. Sensation on palate now gone.

130 minutes. Very fluid dejection quite devoid of formation, accompanied by relief of symptoms except that slight nauseation persisted for an hour longer. No disturbance in night following, urine not distinctly increased in quantity.

18 hours. The following morning evacuation quite unformed, no further effect.

(NOTE. Whilst doses of 1 dcg. and upwards of the seed produced diuresis this ceased to appear when a definite hydrogogue cathartic action supervened. This point is more closely examined in the section dealing with the results of dietetic administration of the seeds to rabbits.)

ACTION OF INGESTED OIL OF CROTON ELLIOTIANUS UPON MAN

0.02 cc. Doses of 0.02 transferred to a lump of sugar which was allowed to melt in the mouth before swallowing and succeeded by 40 cc. water, produced nothing further than slight diuresis with dejecta of softer consistency on the morning following.

0.04 cc. No local effect upon palate. After four hours borborygmi with tendency to evacuation, which passed off. Morning succeeding, dejecta soft.

0.08 cc. Taken at 11 a.m.

3 hours, 30 minutes. No local sensation in mouth but borborygmi with transitory tendency to evacuation.

5 hours. Faint eructation, peculiar taste of oil perceptible.

8 hours, 30 minutes. No discomfort but strong tendency to evacu-

ate. Copious unformed evacuation. No disturbance later, though during night some diuretic effect.

21 hours. Morning succeeding medication, unformed evacuation.

36 hours. Evacuation formed. No further effect.

0.1 cc. Produced in thirty minutes slight discomfort on soft palate (much less than caused by equal dose of the seed) soon disappeared.

75 minutes. Observer felt nauseated, eructation, taste of oil, borborygmi.

115 minutes. Borborygmi frequent, tendency to evacuation increasing.

130 minutes. Three distinct actions followed in course of ten minutes, dejecta very liquid.

145 minutes. No nausea.

175 minutes. Meal taken with appetite. There was no recurrence of action but on the succeeding morning the evacuation was quite unformed. There was throughout an absence of diuresis.

ACTION OF OIL-FREE SEED OF CROTON ELLIOTIANUS

A large sample of the seed powder from which the oil had been extracted was supplied from the Imperial Institute. This powder is of a buff color and has a slightly bitter taste. For experimental purposes it was again triturated and passed through a no. 100 sieve. The action upon the human subject was but slight when contrasted with that of the oil-containing seed or the pure oil.

Ingestion of amounts, progressively increased from 0.05 gram to 0.3 gram was negative in result and the dose was raised to 0.5 gram before a laxative effect of feeble character was developed. 0.75 gram did not occasion any local "acrid" sensation on palate or fauces but borborygmi unattended by discomfort ensued in 4 hours and the dejecta on the following morning as well as on the next day were unformed in character. A slight diuretic action succeeded the ingestion of 0.3 gram of the powder.

FEEDING OBSERVATIONS (RABBITS)

A. Seed of Croton Elliotianus

Healthy full-grown animals were employed in these and succeeding observations. They were kept in roomy cages especially adapted to accurate recovery of excreta in a room maintained at uniform temperature. The daily dietary consisted of 150 grams fresh vegetables and 100 grams corn and bran, the latter mixed with 100 ccs. water. When sufficient uniformity of elimination had been established the seed of Croton Elliotianus was administered in emulsified form (0.1 gram of seed per 1 cc. mucilage of gum acacia) by the mouth and the estimations were continued from day to day. The figures given in the table represent faeces and urine for two days previous to administration of the seed and for a similar period thereafter. The administration took place at

	BEFORE ADMINISTRATION		DOSE OF SEED ON MORNING OF 3D DAY		AFTER ADMINISTRATION	
	Faeces	Urine			Faeces	Urine
	grams	*cc.*	*grams*		*grams*	*cc.*
Observation I						
1st day	63.0	125.0	0.05	3d day	65.0	141.0
2d day	64.0	115.0		4th day	61.0	137.0
Average	63.5	120.0			63.0	139.0
Observation II						
1st day	65.0	121.0	0.075	3d day	70.0	161.0
2d day	63.0	131.0		4th day	68.0	121.0
Average	64.0	126.0			69.0	141.0
Observation III.						
1st day	63.0	127.0	0.1	3d day	62.0	167.0
2d day	58.0	116.0		4th day	70.0	123.0
Average	60.5	121.5			66.0	145.0
Observation IV						
1st day	59.0	131.0	0.15	3d day	66.0	173.0
2d day	65.0	122.0		4th day	73.0	134.0
Average	62.0	126.5			69.5	153.5

about 10 a.m., immediately after collection of the excreta passed during the preceding twenty-four hours.

The dosage of 0.15 gram was the largest tolerated without resulting interference with appetite. After all administrations the faeces were passed in a formed condition and only on two occasions* were they soft in consistence.

B. Oil of Croton Elliotianus

The oil was administered either pure or in emulsified form but as the results obtained by the two methods respectively were similar they will be scheduled together.

	BEFORE ADMINISTRATION		DOSE OF OIL ON MORNING OF 3D DAY		AFTER ADMINISTRATION	
	Faeces	Urine			Faeces	Urine
	grams	*cc.*	*cc.*		*grams*	*cc.*
*Observation V**						
1st day	63.0	124.0	0.02	3d day	68.0	120.0
2d day	70.0	112.0		4th day	64.0	113.0
Average	66.5	118.0			66.0	116.5
Observation VI						
1st day	65.0	128.0	0.05	3d day	71.0	142.0
2d day	61.0	117.0		4th day	58.0	123.0
Average	63.0	122.5			64.5	132.5
Observation VII						
1st day	63.0	114.0	0.1	3d day	60.0	194.0
2d day	59.0	120.0		4th day	61.0	150.0
Average	61.0	117.0			60.5	172.0
Observation VIIa						
1st day	58.0	132.0	0.1	3d day	77.0	125.0
2d day	62.0	125.0		4th day	67.0	160.0
Average	60.0	128.5			72.0	142.5
Observation VIII						
1st day	63.0	105.0	0.15	3d day	64.0	223.0
2d day	67.0	119.0		4th day	69.0	149.0
Average	65.0	112.0			66.5	186.0

* In No. V observation the dose is ineffective, variation recorded being within limit of normality.

These results may be represented in percentage increases or diminutions in excreta after administration of seed or oil as follows: The day of greatest increase is also indicated.

OBSERVATION	SEED OR OIL	DOSE	PERCENTAGE VARIATION				DAY OF MAXIMUM AFTER ADMINISTRATION	
			Faeces		Urine		Faeces	Urine
			+	−	+	−		
I	Seed	0.05 gram		0.8	19.0			1st
II	Seed	0.075	7.8		12.0		1st	1st
III	Seed	0.1	8.0		19.0		2d	1st
IV	Seed	0.15	12.0		22.1		2d	1st
V	Oil	0.02 cc.		0.75		1.2		
VI	Oil	0.05	2.5		8.1		1st	1st
VII	Oil	0.1		0.8	47.0			1st
VIIa	Oil	0.1	20.0		10.7		1st	2d
VIII	Oil	0.15	1.5		66.0		2d	1st

The figures indicate that after effective doses of seed or oil of Croton Elliotianus there is a total increase in urinary and intestinal excretion when estimated jointly but that whilst the urine is invariably augmented the faeces may be diminished in amount. Thus out of ten observations (only 4 of which appear in the schedule) in which the seed was administered there was increased separation of faeces in 8 and reduction in 2 whilst of 12 observations of oil administration (5 scheduled) there was faecal increase in 8 and reduction in 4. When such reduction occurs it is invariably associated with a more evident increase in the amount of urine separated whereas an actual increase of elimination by the intestine is attended by a slighter urinary augmentation (this is well exemplified by observations VII and VIIa; in the former the action is mainly renal whilst in the latter it is intestinal). Thus in the rabbit, between the intestinal and renal elimination as modified by Croton Elliotianus a vicarious balance tends to develop and in this animal the purgative action is much less pronounced whilst the diuretic effect appears to be relatively more evident than is the case in the human subject.

The main augmentation of the urine occurs in rabbits during the first twenty-four hours after administration (in all obser-

vations but VIIa) whilst the maximal separation of faeces in all cases of increase takes place more frequently (in 60 per cent nearly of all observations) on the second day after, rather than on the day of administration. It is probable that intestinal peristalsis, which evidently is readily aroused in man and which will be shown to be speedily produced in cats by Croton Elliotianus, has less action on the thin muscular walls of the lengthy rabbit's intestine and in consequence absorption followed by a direct action upon the renal cells will be more readily induced. Where such early diuresis occurs the secretion from the intestinal mucosa will take place with less freedom and in this manner an actual reduction or only a very limited augmentation of faecal matter may result. That the latter retains its formed character and is only reduced in consistence occasionally is a further evidence of the very restricted cathartic action of the oil or seed towards rabbits.

INGESTION OF OIL-FREE SEED (RABBITS)

The results obtained by feeding rabbits with oil-free powder mixed with the food were much less constant than when the oil-containing seed was employed. Thus 0.1 gram was followed by increase of faeces by 1.3 grams and decrease of urine by 2 cc., which must be regarded as negative as the figures fall within the limits of diurnal variation. The average of five observations in each of which 0.5 gram of the powder was given showed an increase of faeces by 4.25 grams and of urine by 17.5 cc., a moderate but positive reaction.

These results are in accord with the observation that in man the seed of Croton Elliotianus, when deprived of oil, possesses but slight eliminative action (as gauged by faeces and urine) when contrasted with the oil-containing seed.

ACTION OF THE SEED UPON FROGS

Healthy specimens of R. esculenta and R. temporaria were employed in these experiments. Injection was made of the seed very finely reduced, emulsified with gum acacia and water into (a) the stomach, (b) the lymph sac.

a. Gastric administration of doses varying from 0.005 to 0.015 gram produced no immediate effect. The emulsion was not rejected. In less than one-fourth of the observations a slight evacuation of bile tinged mucus occurred within three days of administration but as such evacuations were taking place with similar frequency amongst control animals an inference that the seed of Croton Elliotianus is purgative towards frogs would not be justified. Some increased vascularity of the mucous membrane especially near the pylorus and in the duodenum with increased mucous secretion was observed in certain of the animals destroyed five days after administration of the seed but in none of them were haemorrhages or ulcerations present.

b. When injected in emulsified form into a lymph sac the reduced seed is distinctly, though not necessarily rapidly, toxic. Doses of 0.01 gram prove lethal in five to six days, of 0.002 gram in from three to four weeks, whilst 0.001 gram is rarely lethal. There is neither excitement nor other evidence of irritant action as a sequence to injection of a lethal dose of 0.01 but the animal if destroyed in forty-eight hours after injection exhibits vascular haemorrhage, especially in the dependent portion around the urostyle and haemorrhagic points upon the walls of the lymph sac; elsewhere serous oedema of the thighs and pallor of the tissues, the latter in correspondence with the degree of haemorrhage, are also present. The posterior lymph hearts are frequently arrested. Films of blood stained with Jenner's reagent or examined unstained show endoglobular degeneration of the colored elements and sometimes additional poikilocytosis; such appearances are more marked however when the injection has been longer survived. If the dose is smaller other conditions may arise. The buccal mucous membrane, especially on the palate, (fig. 2, *a* and *b*), may show distinct congestion on the third or fourth day, small raised points containing at first a minute amount of clear fluid appearing subsequently on this area. Later the fluid becomes haemorrhagic. Exceptionally these points invade the mucous membrane lining the rami of the lower jaw; they are rarely seen in the tongue itself. The number and size of these haemorrhagic points vary but increase in both

respects occurs with time. Slight oozing of blood or more rapid haemorrhage may supervene on this condition resulting in anaemia and weakness.

Of less frequent occurrence are the small petechial haemorrhages which may appear in other localities. Especially are these to be found in the gastrocnemii and extensor cruris (fig. 3), the points being in some instances so numerous as to give a "flea-

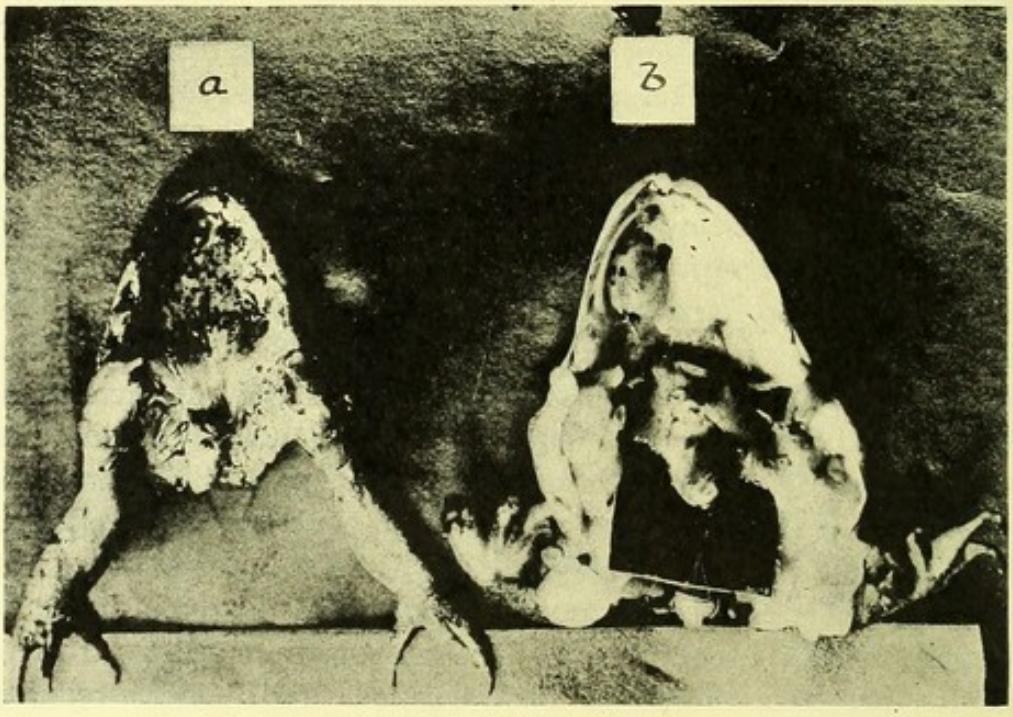

FIG. 2. PALATAL HAEMORRHAGIC SPOTS AFTER CROTON ELLIOTIANUS

Small and numerous in *a*, large and few in *b*. In *b* there are two blood sacs in the ventricular substance.

bitten" appearance. They are also found in the oviducts (fig. 4).

The ventricle usually shows one or more blood-containing sacculations (fig. 2), whilst the lungs and central nervous system are unaffected. Vascular engorgement, occasionally haemorrhages of the pyloric end of the stomach, more usually of the duodenal mucous membrane (fig. 5) may follow injection into the dorsal lymph sac, the blood which is found in these localities may

have an additional source in the buccal haemorrhages already referred to. Microscopical examination of the hardened and stained duodenum in certain cases shows the presence of blood extravasation in the submucosa, in the mucosa and less obviously in the musculature of the intestine (vide fig. 5, reduced from a microphotograph).

Rarely, small vesicular points 0.5 to 1 mm. in diameter, which

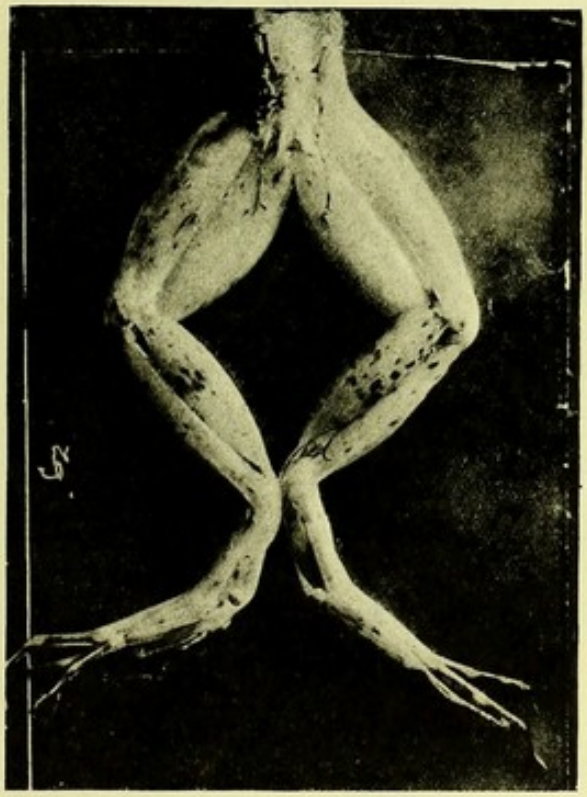

FIG. 3. HAEMORRHAGIC SPOTS CHIEFLY IN BOTH GASTROCNEMII AND IN LEFT EXTENSOR CRURIS

subsequently become haemorrhagic, appear on the skin covering the hyoid and upper sternal regions (these have also been seen by us as a sequence to the hypodermic injection of Kobert's ricin from R. communis). That the buccal haemorrhage proceeds from local vascular necroses is demonstrated by destroying the brain and cord of a frog exhibiting these blood-stained points or spots, inserting a cannula in the aorta, dividing the inferior vena

cava and circulating through the preparation Ringer's solution strongly tinged with methylene blue. Pressure is gradually increased by raising the funnel containing the solution; it is then noted that escape of the colored fluid first at one and then at

FIG. 4. HAEMORRHAGIC SPOTS IN THE OVIDUCTS AFTER CROTON ELLIOTIANUS

others of these palatal spots becomes evident and that at comparatively low pressures.

Neutralisation of the seed has a slight effect in reducing local haemorrhage at the sight of injection into the lymph sac; but it

does not altogether hinder its occurrence whilst it in no way interferes with the remote haemorrhages which ensue so frequently on the injection of the untreated seed. Parallel observations have shown that delay in the lethal issue is by about two days when doses of 0.02 gram after neutralisation are injected whereas delay is increased to approximately three and a half days when double that quantity (0.04 gram) is employed.

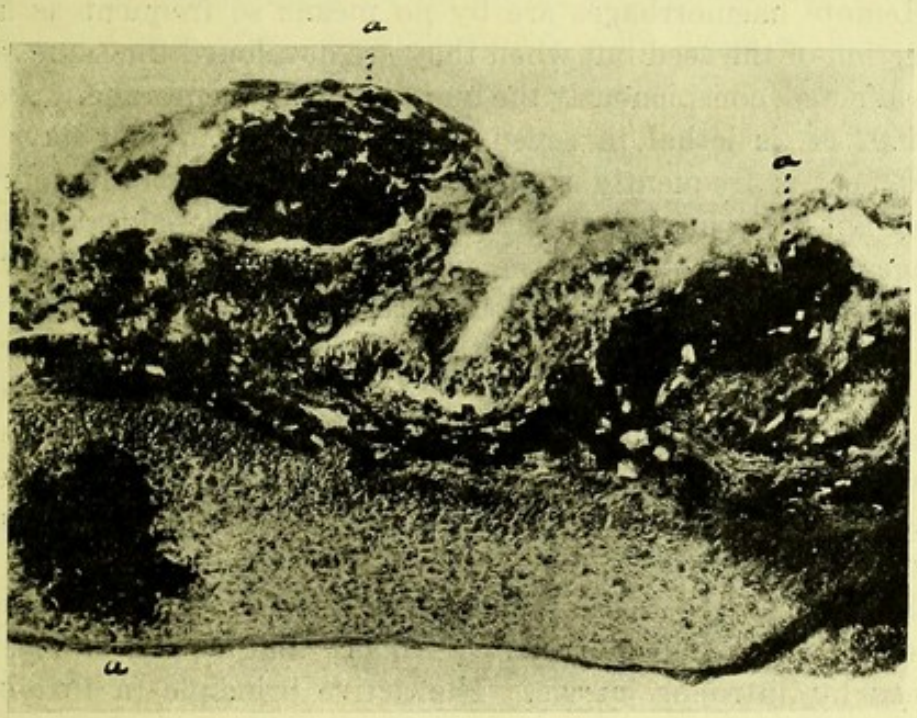

FIG. 5. DUODENUM OF FROG

After injection of emulsfied seed of Croton Elliotianus into dorsal lymph sac. T. S. Enlargement × 30. *a*, haemorrhagic extravasations.

ACTION OF OIL OF CROTON ELLIOTIANUS UPON FROGS

a. Gastric administrations of 0.02 to 0.08 cc. (the latter dose suffices to cause a laxative effect in man) are not rejected nor do they usually cause purgation. Local inflammation of the ventral gastric mucosa was only exceptionally seen whilst remote haemorrhage was recorded in but one observation. Whilst the smaller dose (0.02 cc.) is sublethal to a 30 gram frog the larger may prove lethal in twelve to thirteen days.

b. Hypodermic injection into lymph sac. The oil administered pure or in emulsified form is much more (×4) toxic than is an equal dose given by the stomach, the reason being that local haemorrhage is much more readily induced. Haemorrhage occurs mainly from the vessels in proximity to the posterior part of the urostyle and to a lesser extent on the dorsal wall of the sac. The lymph hearts (posterior) may be arrested.

Remote haemorrhages are by no means so frequent as after injection of the seed but when they are developed the same parts are affected, conspicuously the buccal mucous membrane. A dose of 0.02 cc. is lethal in seven days, of 0.2 cc. in four days but 0.002 cc. is frequently sublethal. Remote petechial spots are more apt to occur where smaller doses causing less local haemorrhages are given. When the tissues are rendered distinctly exsanguine as the result of copious local haemorrhage they are very seldom produced. Dilution of the oil (1) by almond oil or (2) by mucilage does not alter the lethal dose but delays the lethal issue. Exact neutralisation of the oil by means of potassic hydrate or bicarbonate of sodium solutions does not obviate the haemorrhagic action though here also there is delay of a day or two in the final result.

The action of Croton Elliotianus upon frogs bears a close resemblance to that recorded by Kobert[3] and his scholars as produced by Jatropha curcas. The active principle (a toxalbumose "curcin")[4]—occasions remote haemorrhages in the same localities as are involved by Croton Elliotianus.

ACTION OF OIL-FREE SEED OF CROTON ELLIOTIANUS ON FROGS

After injection of the fine flower-like powder obtained from the seeds after complete removal of their oil a toxic effect may still ensue. The powder is however weight-for-weight less active than the seed. Its action qualitatively is the same.

The relative toxicity of (1) fresh seed, (2) extracted oil, and (3) oil-free seed, administered hypodermically to frogs may be rep-

[3] Kobert: Beitr. zur Kennt. d. veget. Hämagglutinine, Heft II, Berlin, 1913. S. 155 et seq.

[4] Ibid., p. 126.

resented by the following figures which represent minimal lethal dosage for each: Fresh seed, 0.002 gram for a 30 gram frog or 0.066 per kilogram; oil, 0.083 cc. per kilogram, and oil-free seed about 0.09 per kilogram.

LOCAL (CUTANEOUS) APPLICATION OF OIL OF CROTON ELLIOTIANUS

Man. Measured quantities of the oil were applied with friction to marked skin areas, re-applications being made at intervals. Thus in observation 4, 0.02 cc. was rubbed on to the back of the hand over an area equal to a florin. No effect resulting, re-application was made daily on four succeeding days to the marked area, 0.1 cc. in all being thus employed. There was no effect, subjective or objective either at the time or subsequently. The observation was repeated with the following modifications: (a) the employment of heat with inunction of the skin area, (b) treatment with weak acid or (c) weak alkaline solutions before application of the oil, but the results were uniformly negative.

Rabbits. The oil was applied to the inner surface of the external ear of three rabbits in quantities of 0.02 to 0.04 cc. with gentle inunction. Re-applications were made at intervals of two to four days upon from four to five occasions. In three observations there was a variation in method: a small pledget of cotton wool into which 0.04 to 0.06 cc. of the oil had been soaked was enclosed within the longitudinally folded ear, the margins of which were held together by plaster. The contact was thus insured for from twenty-four to thirty hours. In none of the six observations was any irritation, increased vascularity or eruption produced; in five there was entire absence of subsequent desquamation. In one instance (of the second series) slight desquamation was detected on removal of the application and persisted for six days.

The oil of Croton Elliotianus must therefore be regarded as devoid of the local irritant action, when applied to the skin, which is so obviously produced by the oil of Croton Tiglium (Croton oil).

HYPODERMIC INJECTION OF OIL

Hypodermic injection of Croton Elliotianus oil appeared essential in order that so employed its action might be contrasted with that of Croton Tiglium. The latter is extremely toxic whether injected hypodermically or into the peritoneal cavity. Thus, Rosenbach and Pawlowsky[5] found that whilst rabbits survived injection of a quarter of a drop of Croton oil diluted with twenty-four parts of mucilage into the peritoneal cavity other animals succumbed within twenty-four hours when four times that dose had been administered. Post mortem, great hyperaemia with haemorrhagic exudation. Larger doses intensified the effect. Dinochowski and Janowski[6] injected Croton oil (1-8 olive oil) under the skin of dogs, producing thereby a localized inflammation with round celled infiltration of the subcutaneous tissue. Fourteen days elapsed before abscess formation took place; the contained pus was free from micro-organisms. This result has been confirmed by subsequent investigators.

Observation 1

Hypodermic injection of the Oil of Croton Elliotianus was made by a sterilised syringe and with antiseptic precautions otherwise.

March 1. Vigorous full-grown rabbit. Rectal temperature 39.9°C. Colored corpuscles 6,900,000, colorless 3000.

12 a.m. Inject under skin of flank 0.10 cc. oil of Croton Elliotianus with 0.4 cc. Ol. Amygdalae.

March 2. No local tenderness nor induration. Appetite unimpaired. Temperature 40.2°C. Colored corpuscles 5,840,000, colorless 4000.

March 3. Temperature 39.6°C.

March 4. There is slight local swelling, no tenderness (nor was there later). Temperature 39.5°C. Colored corpuscles 5,500,000, colorless 9000.

March 8. Slow increase of swelling, which is more indurated but quite circumscribed. Temperature 39.6°C. Colored corpuscles 5,100,000, colorless 13,200.

March 10. Swelling is now the size of a small almond. Temperature 39.6°C. Colored corpuscles 6,800,000, colorless 6400.

[5] Arch. f. Exper. Path., Bd. cxvii, 1889, S. 469.

[6] Ibid., Bd. xxxiv, 1894, S. 105.

March 13. The feeling of the swelling indicating the presence of pus. A puncture was made with a sterilised hypodermic needle, after disinfection of the skin with perchloride of mercury solution. A small droplet of pus was obtained a portion of which was immediately transferred to an agar cultivating tube (which was thereafter incubated) whilst the remainder was treated with Jenner's stain for microscopical examination. The tube remained sterile and the examination for micro-organisms negative. Temperature 39.8°C. Colored corpuscles 7,000,000, colorless 7200.

The further history of this observation will be summarised: The swelling increased, though but slightly, up to the eighteenth day and after remaining stationary for a week there was a slow but progressive decrease in size. No pyrexia. Animal feeding well and weight increasing. The colored corpuscles 6,640,000, colorless between 5000 and 6000.

April 24. Fifty-five days after injection the animal was destroyed.

No abnormalities were detected in the central nervous system, alimentary canal, lungs, heart or kidneys. A small encysted collection of caseous matter was found at the seat of the injection. This cyst, when dissected out and unpunctured, weighed 1.25 grams. A minute portion of the contents was transferred to a cultivating tube, another part being stained as before. No growth occurred after prolonged incubation. The results were, as previously, entirely negative.

Observation 2

Animal full-grown. 0.05 cc. of the oil diluted with nine parts of almond oil, similarly injected.

Epitome. Local effect: Local induration detected on the eighth day, pus present on the fourteenth day, the swelling being oblong 15 mm. by 7 mm. and quite circumscribed. No discomfort.

Blood. The colored corpuscles reduced by 1,000,000, i.e., to 5,500,000 on the seventh day but on the eleventh the number was approximately the same as before injection, i.e., 6,490,000. There was temporary leucocytosis as in the first observation.

Temperature. No pyrexia. Temperature (rectal) never exceeded 39.8°C.

Faeces and urine. The faeces averaged 7 grams more, the urine 13 cc. less for the two days succeeding administration of the oil as contrasted with the two days preceding. The urine was throughout devoid of albumen and blood pigments. In this observation the swelling gradually

decreased until a little fibrous nodule less than a lemon pip in size was all that remained eighteen weeks after the injection. This finally disappeared. The animal remained in excellent condition throughout, its weight increasing by 459 grams during the observation.

From these observations it is evident that whilst the oil of Croton Elliotianus does not produce local irritation when applied to the skin, thereby differing from the oil of Croton Tiglium, it does like the latter, even when largely diluted, occasion a slowly developing suppurative process independently of microbic action. The extent of reduction in the number of colored corpuscles as well as the degree of leucocytosis appear to bear a direct relationship to the amount of oil introduced into the subcutaneous tissue.

Although neither appearance nor reaction of the urine in the first three observations had revealed the presence of blood pigments it seemed probable that the large reduction in the number of the colored elements of the blood following hypodermic injection of the oil of Croton Elliotianus would, at least occasionally, be followed by elimination of blood pigment. There was also the further possibility of nephritis owing to irritation during elimination. Two observations were therefore made in which the urine, before and after injection, was repeatedly examined by the spectroscope, simultaneous blood counts being recorded, one of these in harmony with the first three was altogether negative in its results, the second—which is tabulated below—was positive.

This observation indicates a marked reduction of erythrocytes and a coincident appearance of haematoporphyrin with a trace of albumen in the urine. The nature of the pigment as well as the small amount of albumen eliminated and the very short duration of albuminuria, with total absence of tube casts, seem to indicate that the conditions observed are referable to haemolysis rather than to the induction of nephritis.

Observation 5. (*Diagram 1*). *Rabbit* (*full grown*)

DAY OF OBSERVATION	HAEMOGLOBIN	COLORED CORPUSCLES	LEUCOCYTES	URINE	NOTES
	per cent				
−5	78	7,440,000	5.33		Blood taken 2 hours after feeding.
−1	80	7,716,000	6.16	No abnormality of urine.	
0 12.30 p.m.					12.30 p.m. inj. 0.1 cc. Ol. Crot. El. in .9 cc. almond oil.
1 11.30 a.m.	78	8,720,000			
1 3.00 p.m.		8,495,000	6.40	Dark brown, acid, phosphates. No albumen. No blood pigment. Spectrum urobilin.	Some poikilocytosis.
2				Urine dark, concentrated 40 cc. Specific gravity 1040; trace of albumen. Spectrum haematoporphyrin. Peculiar form phosphates. No tube casts.	
3	70	6,536,000	6.76	Urine dark porter colored. 38 cc. acid. Specific gravity 1044. Haematoporphyrin. Albumen Phosphates. No casts.	Dyspnoea on movement.
4				Character as on 3d day.	
6	65	5,576,000	10.50	Urine dark and specific gravity 1036. Haematoporphyrin (acid). No albumen.	Slight Dyspnoea.
8	75	7,344,000		Urine dark, acid. Specific gravity 1020. Albumen slight trace. Spectrum Acid. Haematoporphyrin.	

Observation 5—Continued

DAY OF OBSERVATION	HAEMOGLOBIN	COLORED CORPUSCLES	LEUCOCYTES	URINE	NOTES
	per cent				
11				Spectrum of haematoporphyrin.	
14				No spectrum.	Small knot-like tumour, size 5 by 4 mm. at seat of injection
16	83	7,420,000	5.70	No spectrum.	
22	85			No spectrum.	Tumour firm. Size small almond.
30	80	7,864,000			

Observations on intestinal movement occasioned by injection of seed or oil of Croton Elliotianus.

The cats which were used in these observations had been fed on milk and meat up to the day preceding but on the morning of the experiment milk only was offered. Ether was the anaesthetic employed. The body temperature was maintained by enclosing the animal in a box, the double walls of which contained hot water or else by partly immersing in water kept at the required heat (the surface of the water was necessarily below the level of any incision requisite for the preparation of splanchnic oesophageal nerves, etc.), sensitive rubber bags having a diameter of 7 to 8 mm. (when gently distended) and a length of 10 to 12 mm. secured on stems of fine gum elastic catheter were used for the reception of contractile movements from the circular muscle coat of the small intestine, a correspondingly larger size being substituted for colon or rectum. Introduction of the sound into the duodenum by way of the pylorus was effected through an opening made within a small ligatured area on the anterior wall of the stomach about 2 cm. from the pyloric orifice. A very fine rubber tube was attached to the stem of the sound for carrying injections directly into the lumen of the intestine at a point having a known distance from the compressible bag. These were secured in position by knotting together threads passing from the stem of the sound with those at the margin of the ligatured tissue. When the lowest portion of the duodenum or jejunum was to be subjected to examination the sound was passed into position from an opening made

below in the course of the intestine. The ileum was reached by passing the sound from the caecum through the ileo-caecal valve, the proceeding being reversed when movement of the colon was under examination. Occasionally a second sound was introduced into the last portion of the colon by way of the rectum. Wherever situated the small open-

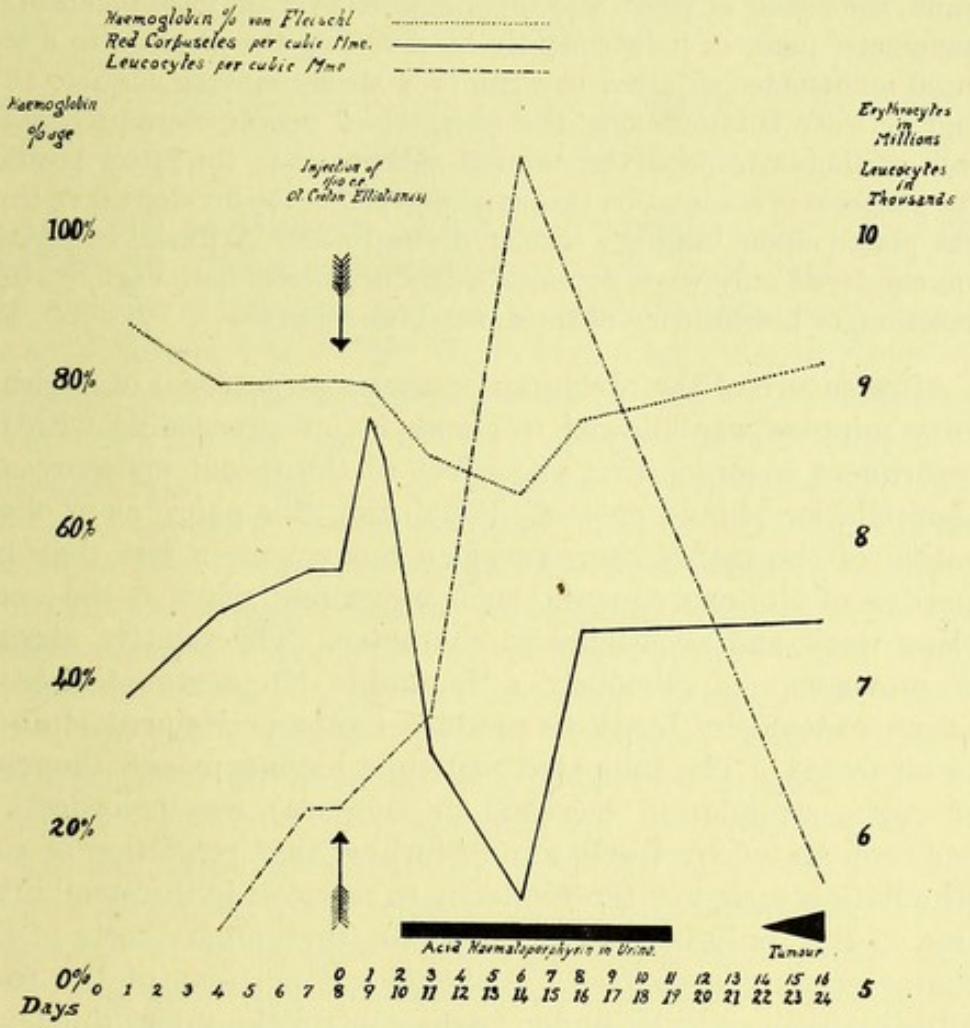

DIAGRAM I

Estimation of haemoglobin ..... Colored corpuscles, and Leucocytes before and after hypodermic injection of Oil of Croton Elliotianus.

ing which had served for the admission of a sound was carefully closed around the stem of the latter, the intestine returned to the abdominal cavity and the incisions in linea alba and skin accurately sutured around the emerging tubes. The sounds were now connected with delicate

recording mercurial manometers by means of thick walled rubber and intervening composite metal tubes, the whole system being filled with water in order to distend the bags and produce a positive pressure of 4 mm. of mercury in the manometers. Blood pressure was derived from the right carotid artery and recorded by a straight spring Fick's kymograph, the needle of which was superimposed over the glass points of the manometer pens, or independently by diverting the pressure to a mercurial manometer adjusted to write on a slowly moving surface. The vagi or their continuations, the oesophageal nerves, were prepared in neck or thorax respectively, the left splanchnic in the latter position. For purposes of stimulation the peripheral end of the divided nerve trunk was placed upon Ludwig's insulated electrodes. Artificial respiration was employed only when necessitated by division of both vagi, by costal resection, or insufficiency of the natural function.

After suture of the abdominal parietes an interval of, at least, forty minutes was allowed to elapse before proceeding with the experiment in order that the effect of the recent exposure and manipulation should pass off; but during this pause close observation of the manometers revealed movement in less than one quarter of the experiments, such movement when it did occur being weak and occasional in character. The relative absence of movement is obviously a favorable circumstance when the action of agencies likely to produce motor excitement is about to be tested. The indicated rest-time having passed the result of vagus stimulation (cervical or thoracic) was recorded. It has been stated by Bayliss and Starling[7] that repetition of such stimulation increases the tendency to reaction in the small intestine so that only one, or at most two, preliminary tests of this character were permitted. Although all portions of the small intestine appear to be under vagus control the upper(duodenal) portion is peculiarly so. In four of the experiments of the present series the duodenum was not under observation but out of the remaining thirteen in which it was, in two only was a registrable effect produced by this initial vagus stimulation. Injection of the emulsified seed or (alternatively) of the oil of Croton Elliottianus warmed to body temperature was made from a hypodermic

[7] Journ. of Physiol. Proc., November 12, 1898, p. 8.

syringe (previously heated) by way of the fine rubber tube attached to the stem of the sound. The mouth of this tube within the intestine was situated at a measured distance from the compressible bag; the free (external) end was clamped by light spring forceps after injection had been made. The actual bulk of the injection varied from 0.5 to 1. cc.

MOVEMENT FOLLOWING INJECTION OF EMULSIFIED SEED OR OIL OF CROTON ELLIOTIANUS

After the introduction of 0.1 to 0.2 gram of gum emulsified seed ten to fifteen minutes elapse before movement occurs in the segment of intestine in which the compressible bag is situated at a distance of 4 to 5 cm. When first observed such movement is feeble, occasional and unsustained in character. The phase of greatest spontaneous activity is attained in from twenty-five to thirty-five minutes after injection, the contractions being at that time energetic and sustained in character. But the condition is only transitory for the excitement begins to abate in from forty to forty-five minutes and gradually quiescence is established. A slight tendency to contraction may exceptionally persist up to sixty or seventy minutes. Larger doses cause a more obvious maximal effect of somewhat longer duration. Contractions of the circular coat take the form of groups in which active waves, sometimes superimposed one upon another but more often single, pass over the wall of the intestinal tube whilst during the intervening relaxations it is often evident that the muscular fibres maintain some degree of tonic contraction. Following such manifestations of excitement there is a longer or shorter interval of rest before activity is renewed. At the height of action the groups are more frequent in occurrence, the contractions more vigorous and persistent, whereas towards the commencement and end of excitement the group character yields to that of occasional waves which are followed by complete relaxation. Atropine in small or medium doses does not check these manifestations.

As contrasted with the seeds (which may show individual

variation in their activity) the oil acts more uniformly but the local excitement it occasions is less, the groups of contractions are not so sustained and the intervening periods of quiescence are longer.

Very large doses of the oil may cause prolonged contractions, having an almost uninterrupted character. Thus (fig. 6) the injection of 0.2 cc. of the pancreatised oil into the duodenum causes a powerful response lasting for forty seconds but subsequently groups of wave-like contractions only were recorded.

Whatever may be the degree of motor reaction to a primary administration of seed or oil repetition of an equal dose after the phase of activity has passed is distinctly less effective but if the injection be repeated whilst excitement still prevails this is increased. Even when the phase of movement has given place to rest the intestinal wall will contract vigorously to (a) a larger dose of the oil or seed or (b) the intravenous injection of a small amount of nicotine or (c) of chloride of barium.

From examination of the results obtained by repetition of similar (medium) doses of seed or oil at varying intervals of time it appears that if two hours or longer elapse between two injections the degree of response will be much the same on each occasion.

The large intestine was similarly examined. Two sounds were usually employed, one introduced through the wall of the ileum by way of the ileo-caecal valve into the colon, the second passed upwards from the rectum into the lower portion of the colon; the upper sensitive bag at the extremity of the sound was usually adjusted in position so as to be about 6 cms. within the valve whilst the lower was from 8 to 10 cm. on the rectal side of the former. The emulsified seed or oil was introduced into the colon through a fine rubber tube attached to the stem of the upper sound so that its discharging end was at a known distance from the recording area. A full dose of emulsionised seed, i.e., 0.15 to 0.2 gram caused in from ten to fifteen minutes distinct contractions in the upper part of the colon, the excitement being speedily communicated to the segment within which the second recipient bag was situated some 10 to 12 cm. below.

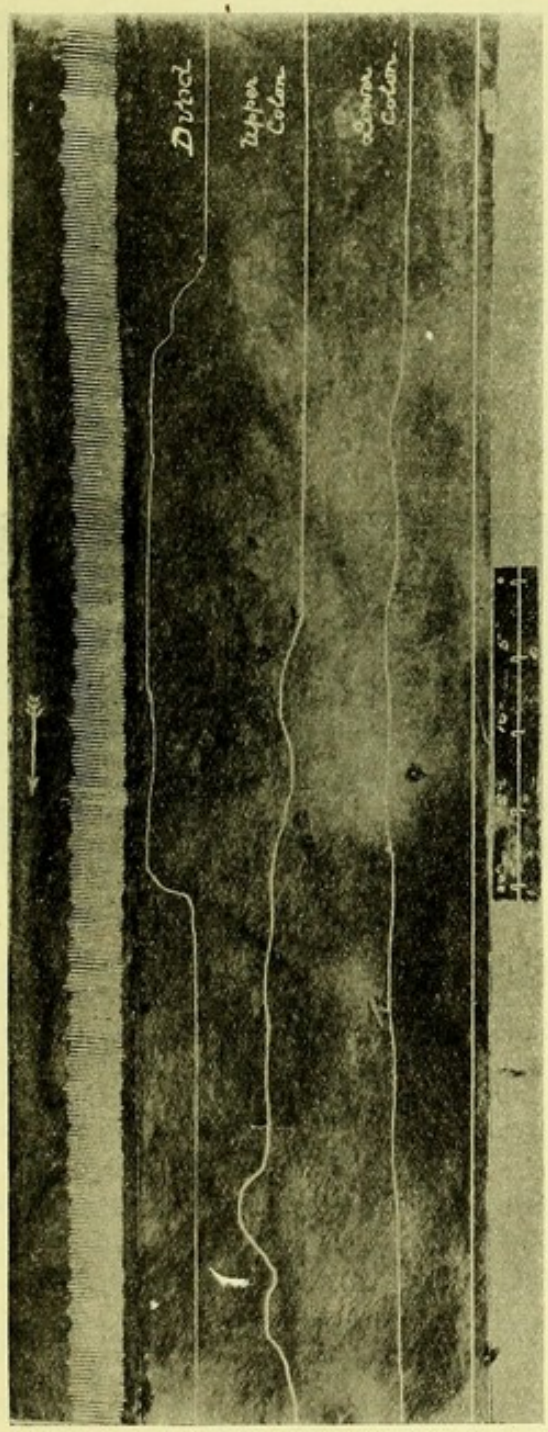

FIG. 6. CONTRACTIONS OF DUODENUM AND COLON AFTER OIL OF CROTON ELLIOTIANUS

Two separate injections of 0.2 cc. of pancreatised oil had been made, No. 1 into the duodenum thirty minutes before, No. 2 into the upper colon 4 cm. above the sensitive bag forty minutes before. The lower colon registration is at a point 11 cm. below that of the upper.

The maximal motor effect was recorded about forty minutes after injection, the contractions thereafter though but little diminished in point of frequency were less sustained in character. In from seventy minutes to eighty minutes the intestine was quiescent. The contractions recorded by the two manometers connected with the compressible bags differed in this respect that the movements near the ileo-caecal valve were of a less interrupted and more persistent character whilst those nearer the rectum exhibited a stronger tendency to intercurrent relaxation. This difference is in part referable to a more concentrated condition of the injected material in proximity to the upper bag. Figure 7, from a tracing taken forty-five minutes after injection

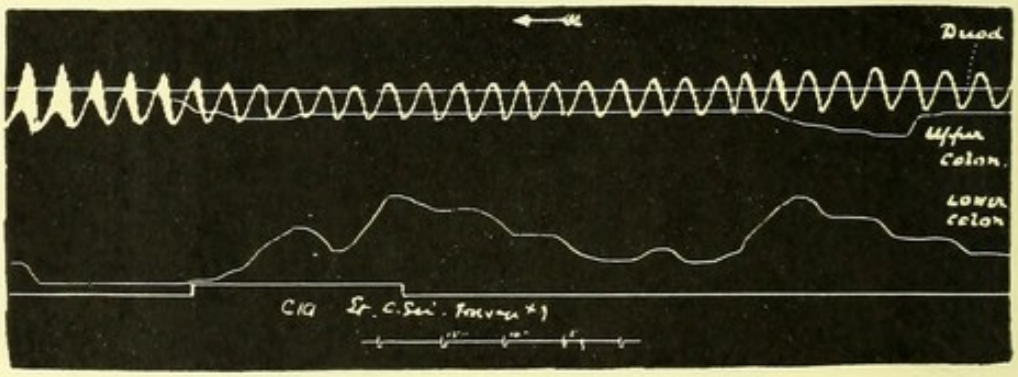

FIG. 7.

Top (horizontal) line, duodenum (uninjected), second line, upper colon; third line, lower colon. Injection of 0.15 gram emulsified seed of Croton Elliotianus had been made into colon (4 cms. above upper bag) forty-five minutes previously. Both vagi are cut. Central sciatic stimulation appear to suspend movement in lower colon for a few seconds. Time marker records intervals of five seconds. Drum accidentally checked for an instant at position marked with x.

of 0.15 gram seed of Croton Elliotianus into the colon, illustrates this point. See also figure 6.

VAGUS STIMULATION AFTER INJECTION OF CROTON ELLIOTIANUS SEED OR OIL

For purposes of stimulation a Leclanché element was placed in circuit with the primary coil of a du Bois Reymond's inducto-

rium. A mercury key for making and breaking connection with the electrodes was included in the secondary circuit, serving by independent contacts to operate simultaneously the electrical signal. The position of the secondary coil at which the faradic current was perceptible to the tip of the tongue having been ascertained, approximation by 2 cm. towards the primary was made for vagus stimulation, the actual distance separating the coils being from 12 to 14 cm. Preliminary stimulation once repeated elicited response in the duodenum in no more than two out of thirteen observations but when introduction of the warmed emulsified seed or oil had been made it was observed that stimulation made in from seven to ten minutes thereafter was almost invariably followed by a series of contractions of the circular intestinal coat.

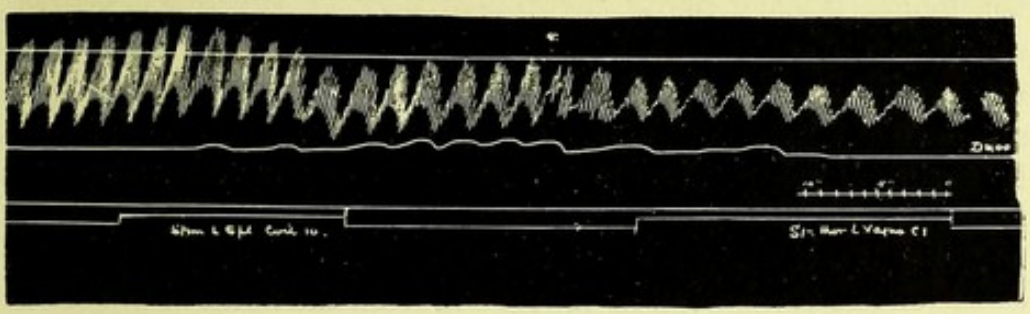

FIG. 8. DUODENAL RECORD

Anterior oesophageal (intrathoracic) stimulation eight minutes after injection of 0.15 cc. Oil of Croton Elliotianus into lumen 4 cm. above compressible bag. Stimulation before injection inoperative.

Exceptionally response was delayed beyond the 10 minutes interval. Such responses are not attributable to repetition of stimulation and they are usually to be elicited before any independent contractions have occurred as the result of the injection. But when the latter have supervened it is found that in the intervening periods of quiescence vagus stimulation causes the phenomenon of group contractions with great regularity. If such stimulation is purposely made during spontaneous activity this may be weakened for a brief period and then intensified and prolonged; exceptionally no initial weakening is recorded.

Such effects were produced equally by appeal to the left cervical vagus or to the anterior oesophageal nerve. They were not interfered with by small, but were reduced or altogether abolished by large, doses of atropine. They were unaffected by ligature of the undivided (right) vagus. The latency intervening between commencement of stimulation and motor effect has occasionally been from twenty-two seconds to thirty seconds (in near correspondence with the observations of Bayliss and Starling[8] but much more frequently the interval is no more than seven to ten seconds. Thus the contractions representing a first response to vagus stimulation begin in eight seconds in figure 6, the record being obtained eight minutes after the injection of 0.15 cc. oil of Croton Elliotianus into the lumen of the intestine (duodenum) 4 cm. above the compressible bag. Whilst the longer latency above mentioned may be shortened on frequent repetition of stimulation as has been already mentioned by Bayliss and Starling, the briefer latency undergoes little abridgement from such repetition. One point affecting the prolongation of latency has presented itself so frequently however that it seems advisable to mention it in this place: It is that, whilst a latency of eight to ten seconds is recorded when the sensitive bag is removed by only 4 cm. from the point of injection, if these are further apart (say, 8 or 9 cm.) there is a material increase in the time elapsing between commencing stimulation and movement. This seems to indicate that motor response to vagus stimulation may first appear in an area of the intestine which is already in a condition of excitement as the result of the presence of irritant bodies such as the seed or oil of Croton Elliotianus and that from this area by the agency of Auerbach's plexus a peristaltic wave may pass downwards. We do not offer this explanation as fully proved but rather as being probable. Small doses of atropine do not materially hinder response to vagus stimulation (after the injection has been made into the intestine) but large doses effectually do so. The intestine may continue to show spontaneous contraction after an amount of

[8] Journ. of Physiol., xxiii, p. x, November 12.

atropine which renders vagus stimulation inoperative. It is observed however (in absence of atropine) that during the period of induced activity the responses in the intervals of rest are steady to vagus stimulation but as excitement passes and contractions fail or become feeble and infrequent the vagus effect tends also to disappear. Should a second injection of the seed or oil be made at such an interval after the first (ninety minutes or more) as to cause a recurrence of contractions it is usually (though not invariably) found that the response to vague stimulation again becomes active.

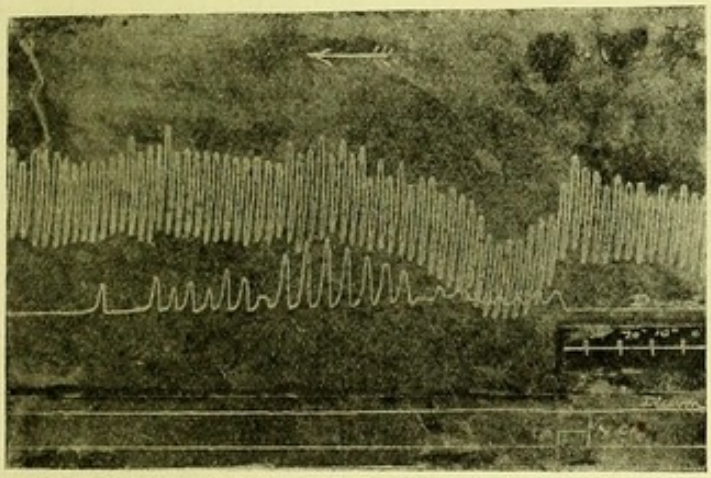

FIG. 9A.

FIG. 9B.

FIG. 9.

A. Upper sound, in duodenum (from stomach); lower, in ileum (from colon). Injection of 0.1 gram. emulsionised seed of Croton Elliotianus 4 cm. above former twenty minutes before. There was no response before injection to vagus stimulation, now operative.

B. Taken seventy minutes later and eighteen minutes after injection of 0.1 gram of seed 4 cm. above iliac sound. The ileum responds to vagus stimulation, The duodenum does not. The time marker indicates ten second intervals.

Lower portions of the small intestine respond in the same way as the duodenum to vagus stimulation though with less certainty. In the experiment from which figure 9*A* is taken powerful contractions of the duodenum follow stimulation of the cervical vagus twenty minutes after the introduction of 0.1 gram emulsionised seed of Croton Elliotianus; the ileum showing very slight

increase of tonic contraction. Figure 9*B* is taken seventy minutes later, a second injection having been made eighteen minutes previously, this time into the ileum, of 0.1 gram emulsified seed. The ileum now contracts actively but the duodenum remains quiescent on vagus stimulation (in this experiment the intestine was divided in its continuity 12 cm. above the lower sound).

Whilst contractions of the circular intestinal coat, whether caused by the introduction of Croton Elliotianus seed or oil or provoked by vagus stimulation after such introduction, occur in deep anaesthesia it is not found that section of the second vagus nerve in any way affects these phenomena. It is conceiv-

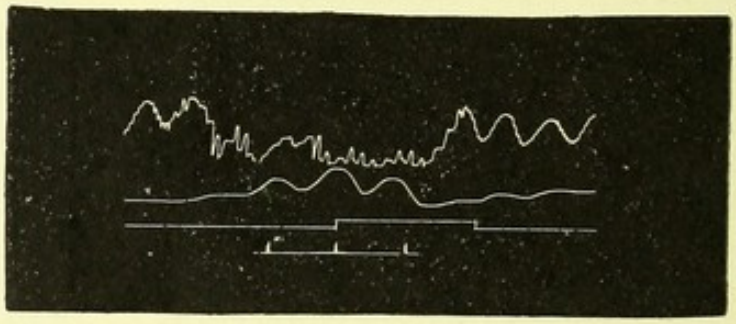

FIG. 10. REGISTERING SOUND IN DUODENUM

Contractions in progress twenty minutes after injection of 0.2 gram Oil Croton Elliotianus when stimulation of left vagus (cervical) is made. Time marker indicates five seconds.

able however that in absence of anaesthesia impulses originating in or reflected from the vagus centre might prove effective in developing or augmenting movements of contraction in an area of the intestine already excited and predisposed to contraction by the presence of such bodies as the seed or oil under consideration. Many influences might in this manner prove contributory to the action of such purgative bodies.

If stimulation of the vagus falls during the prevalence of intestinal contraction caused by the introduction of Croton Elliotianus seed or oil there is usually witnessed a slight check or partial relaxation followed by increased activity of contraction (fig. 10) and if the latency of this positive reaction is measured

it is found to correspond very closely with that recorded when stimulation is made during a period of quiescence.

It is evident from the foregoing results that the increased excitement of the intestinal vagus by the local action of the seed or oil must prove a positive factor with regard to peristalsis and ultimately to purgation. The increase of spontaneous activity after the introduction of these bodies into the lumen of the intestine is no doubt caused reflexly through the intervention of Auerbach's plexus and the ganglia in association. Is it through this channel that the vagus acts? It is probable that it is; at any rate there is no feature in the character of contraction which is exclusively developed by one agency or the other and therefore it is likely that they are referable to one and the same mechanism which is excited directly by the vagus or otherwise reflexly. As has been already remarked small doses of atropine do not materially interfere with the vagal connections with Auerbach's plexus but large ones do so whilst leaving the reflex mechanism intact. The vagus connections are therefore in no way essential for the effective operation of this mechanism. It is however instructive to note that there is after the introduction of Croton Elliotianus a motor effect produced to which the excitability of the intestinal wall to vagus stimulation may show in point of time of appearance, duration and disappearance, a very close correspondence. The return of contractions re-induced by a second injection may also be accompanied by renewed activity of the intestinal vagus.

SPLANCHNIC STIMULATION

Peripheral stimulation of the splanchnic seldom fails to suspend temporarily contractions of the circular intestinal coat resulting from the introduction of the seed or oil of Croton Elliotianus, as well as of those due to subsequent vagus excitation. In two observations absence of inhibitory effect was noted when powerful contractions resulting from the introduction of the seed were at their maximum but this result was of brief duration. Short of this, incomplete relaxation of the

intestinal circular muscle with interruption of the periodic active contractions may be the extent of response to splanchnic stimulation, the intestine resuming its activity immediately afterwards (fig. 11).

Stimulation of the central end of a nerve containing sensory fibres (both vagi and one splanchnic being already divided) usually reduces movement induced by such injections of Croton Elliotianus into the intestinal lumen, but the extent of this effect

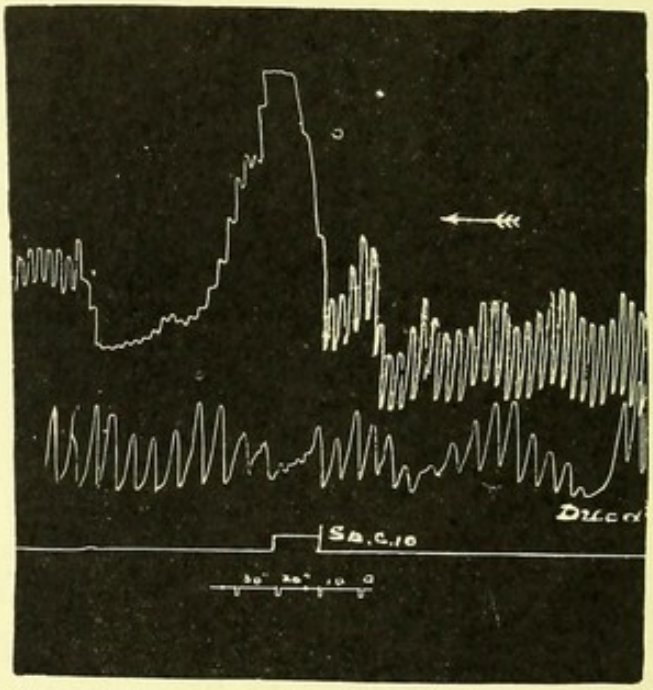

FIG. 11. REGISTRATION (DUODENAL) OF ACTIVE CONTRACTIONS

Twenty-four minutes after injection of 0.3 gram emulsionised seed of Croton Elliotianus into lumen. Splanchnic stimulation (coil 10) has very slight and transitory action, only. Time signal indicates intervals of one second.

is inconstant under variation in the depth of the prevailing anaesthesia. It is yet more uncertain with regard to the movements of the large intestine: thus, figure 7 shows arrest of movement and relaxation of the lower colon following stimulation of the central end of the divided sciatic whilst there is but the slightest relaxation of the powerfully contracted upper colon 5 cm. from the ileo-caecal valve.

The foregoing description of results will now be followed by the record in detail of two experiments:

Experiment 6. Medium-sized cat. Fed (meal and milk) up to day before; on morning of experiment milk only. Ether. Artificial respiration. Left splanchnic and anterior oesophageal (left vagus) nerves, prepared in thorax and placed on electrodes. Cannula in right carotid. Placed in warmed box. Upper sound in duodenum (introduced through stomach) bag 8 cm. from pylorus, mouth of injection tube 4 cm. above. Lower sound introduced from ileum through ilio-caecal valve, bag lies 5 cm. beyond in colon. Abdomen sutured.

Forty minutes later (during which interval duodenum quiescent) twice at intervals of five minutes stimulated vagus, coil 14 and then 12 (current perceived on tongue at 12 cm.). No contraction.

0 minutes. Injected 0.1 cc. warmed oil Croton Elliotianus into duodenum. Blood pressure 100 mm.

10 minutes. Hitherto no movement. Stimulate vagus (coil 14) after 7 seconds active peristalsis in duodenum commenced.

12 minutes. Occasional spontaneous contractions.

22 minutes. Occasional movement, vagus stimulation (coil 14) caused peristalsis (latency 8 seconds), controlled by splanchnic.

32 minutes. Spontaneous movement continues but vagus effect distinct in 7 seconds arrested by splanchnic.

40 minutes. Injected 0.1 cc. pancreatised oil. Duodenal response to vagus stimulation. Contractions last thirty to forty seconds.

62 minutes. Spontaneous contractions of duodenum slightly checked at first then reinforced by vagus stimulation. Colon contracting for the first time during observation.

80 minutes. Stimulation of vagus effective on duodenum but spontaneous contraction has ceased.

92 minutes. Vagus stimulation inoperative—colon quiescent. Blood pressure 86 mm.

NOTE: The second injection of the same amount of oil but predigested with pancreatic extract resulted here in more active response of the intestine than is usual after repetition of the untreated oil.

Experiment 9. Preparation as in preceding but the left vagus exposed in the neck and proximal end of sciatic in the thigh for stimulation. The sound, passed through the pyloric orifice, was sunk deeply in the duodenum, the compressible bag being 11 cm. distant from mouth of injecting tube. During thirty-five minutes observation after suture

of the abdominal wall the intestine was quiescent. After forty and forty-five minutes respectively two stimulations of left cervical vagus (coil 13) caused no intestinal response. Pulse 155. Blood pressure 144.

0 minutes. Injected 0.15 cc. warmed pure oil of Croton Elliotianus into duodenum.

8 minutes. No movement hitherto, on stimulating vagus (coil 13) now distinct contractions after twenty-five seconds interval.

18 minutes. Spontaneous peristalsis in duodenum.

35 minutes. Vagus stimulation (coil 13) causes response in twenty seconds. Pulse 160. Blood pressure 140.

46 minutes. Again effective after same interval (twenty seconds).

50 minutes. Spontaneous peristalsis has now disappeared; at no time was it very active.

65 minutes. No intestinal vagus effect.

80 minutes. Injected into duodenum the large dose of 0.3 cc. warmed oil.

94 minutes. Spontaneous peristalsis.

102 minutes. Response of duodenum to vagus stimulation in nine seconds.

113 minutes. A long continued series of contractions to above in nine seconds. Pulse 166. Blood pressure 150.

In this experiment a considerable length (11 cm.) of intestine intervened between the point of injection and the compressible bag. Responses to vagus stimulation, after the primary injection, occurred in from twenty to twenty-five seconds but after the second administration of a larger dose the latency was of nine seconds only.

ACTION OF CROTON ELLIOTIANUS SEED AND OIL ON INTESTINAL SECRETION

Three equal lengths of small intestine in the lower jejunal and upper iliac regions were separated by double ligatures, a cannula being inserted into each for injection and subsequent collection of fluid, if any were present. Into Section A was introduced 0.1 gram seed emulsified to 1 cc. with gum acacia and water, Section B 0.1 cc. oil emulsified to 1 cc., Section C being reserved as a control. The intestine was then returned to the abdominal cavity. At the expiration of ninety minutes (A) contained 4 cc. of fluid (B) rather less than 2 cc., whilst

the control loop (C) was empty. There is therefore in the cat's intestine as in the human, augmentation of secretion from the presence of both seed and oil in the lumen, the seed causing the greater secretion. Such secretion may be passed onwards under ordinary circumstances or it may be reabsorbed. In an experiment exactly similar to the one just quoted in other respects an interval of 160 minutes was allowed after injection before the observation was completed by estimating the contents of the loops. All three sections were empty, even the small amount of fluid originally introduced (1 cc.) had disappeared from A and B, together with the secretion which presumably had been separated at an earlier stage.

LOCAL ACTION OF CROTON ELLIOTIANUS OIL ON INTESTINAL MUCOUS MEMBRANE

After the conclusion of experiments in the course of which the seed or oil had been introduced into the intestine careful inspection was made of the mucous membrane at the point of contact. In about 77 per cent there was no evidence of irritant effect; in the remainder there was slight vascular engorgement in 18 per cent and marked engorgement in 4 per cent (in each of the latter repeated administration had been made). In one instance only was there erosion with slight haemorrhagic extravasation into the mucous membrane. This exceptional result followed the injection of two 0.1 cc. doses of oil which, owing to over-distension of the compressible rubber bag, had remained entirely above it, thereby producing a local effect which is not developed by temporary contact. The general result is in sharp contrast with that which follows injection of much smaller quantities of Croton Oil (from Croton Tiglium) for in control experiments in which the latter was employed it was found that inflammation of the mucosa with deep vascular engorgement and extravasation extending some distance downwards from the site of introduction were produced. The irritant action of Croton Oil (Croton Tiglium) therefore very greatly exceeds that of the Croton Elliotianus towards the intestinal mucous membrane.

Activity of the oil of Croton Elliotianus as affected by (a) emulsification or by predigestion, (b) with bile, or (c) with pancreatic extract

When a second and equal dose of the pure oil is given within twenty to thirty minutes of the termination of the effect produced by the primary injection it is observed that the result of the former is less than the latter; but if a larger dose be given on the second administration or if the oil is by any means rendered more active then this relationship may be reversed. This information has been applied in determining the relative activity of the pure oil to (a) emulsified oil, (b) oil predigested with bile or with (c) pancreatic extract.

The results obtained are here summarised:

(a) Emulsified with twice its bulk of mucilage of gum acacia the oil is somewhat less active in causing intestinal contraction than is an equivalent of unemulsionised oil.

(b) Preliminary digestion of the oil with cats' bile slightly enhances the action of the former upon the intestine.

(c) Predigested with alkalised pancreatic extract of cat the activity of the oil is distinctly increased (see experiment 6, p.37).

Action of the seeds and oil of Croton Elliotianus upon bile secretion

In two experiments the amount of bile separated was determined by the usual method of collection before and after the introduction of the seed into the lower portion of the duodenum; in a third observation the separated oil was employed. Though in each instance collection was continued for over two hours after administration had taken place the result was uniformly negative, no cholagogue action supervening.

Anthelmintic action

Towards nematode and cestode parasites which occur abundantly in the small intestine of the cat, oil and seed of Croton Elliotianus possess some anthelmintic action. In portions of the intestine into which injections had been made Taenia

crassicollis was found dead (in one observation) and barely living several times, whilst the round worms showed reduced activity as contrasted with those present in other parts of the intestinal tube remote from the injection. The vermicidal action was observed only where strong preparations of the seed or oil had acted. That the action was not energetic when more dilute preparations were employed was demonstrated by placing (1) a tape worm and (2) round worms just removed from their habitat in beakers containing 0.2 gram of finely reduced seed to 10 cc. of warm tap water and transferring to an incubator. The cestode survived for two hours and the nematodes five hours fifty minutes, at which time observation terminated.

It may be inferred from this well as from foregoing experimental data that whilst large doses of seed or oil would occasion such active peristalsis as might cause a vermifugal effect a directly toxic or vermicidal action could not be anticipated as a sequence to such doses of the oil or seed as could be suitably administered to man.

On urinary secretion

As already stated injection of Croton Elliotianus seed or oil may promote diuresis. So slowly however is this effect developed that its demonstration within the time to which a blood pressure observation is necessarily restricted must be imperfect. In four experiments estimation of the urine directly collected from the bladder was made before and after administration of Croton Elliotianus seed by the intestine, the blood pressure being meanwhile recorded. (None of these observations was continued for more than three and a half hours.) In three out of the four there was no appreciable diuretic action whilst in the fourth the result was positive, an increase of secretion to the extent of 16 per cent commencing forty minutes after administration of 0.2 gram of the emulsified seed by the duodenum and persisting for two hours. There was no elevation of the general blood pressure in this experiment nor indeed in the other three which yielded a negative result. The onkometer was employed in two

further observations but in neither was there evidence of increase of the renal volume. After feeding experiments in which the seed or oil respectively has been repeatedly administered no sign of the induction of nephritis has ever been observed either in the urine whilst the animal was living or in the kidney structure subsequently. If the occurrence of diuresis is the result of absorption from the intestinal lumen as is no doubt the case the experimental facts indicate that it is neither the result of increase in the general blood pressure nor of local dilation of the renal vessels. The action is therefore probably exerted directly upon the renal cells but it does not seem to be associated with material increase of soluble salts, the specific gravity being reduced in correspondence with the increase in volume of urine. In man the renal secretion is devoid of irritant action towards bladder and urethra.

Rabbits show—relatively to man—a lower level of susceptibility to the purgative properties of the seed and hence there is greater opportunity for (1) absorption of fluid separated by the intestinal mucous membrane or (2) absorption of the diuretic principle or principles contained in the seed or (3) both conditions may be contributory. In the human subject no diuresis is observed when the dose of Croton Elliotianus suffices to cause hydrogogue purgation, but it may be if the dose is so far reduced that nothing more than a laxative effect follows after many hours.

(a) Blood pressure, pulse and (b) Respiration

(a) No marked action upon the blood pressure and pulse was produced within two or two and one-half hours of the time at which intestinal administration took place, the pressure remaining consistently good and the pulse steady and regular throughout (see notes of Experiment 9). Intravascular injection of seed or oil is naturally inadmissible but injection of filtered extractions of seed (made with (1) Ringers solution and (2) 0.9 per cent saline) were practised. Such extracts were warmed after twice filtering to 40°C. and transfused gradually into the femoral vein. These extracts were inert towards the mammalian

circulation at least within the time limit of a blood pressure experiment. Perfusion of the frog's heart (apical two-thirds) was likewise negative in result when the Ringer's seed extract was used.

(b). Respiration was unaffected by administrations of seed or oil by the alimentary canal but it was slowed and ultimately arrested by intravenous injections of large amounts of extracts prepared from the seed with Ringer's solution. After such arrest artificial respiration failed to restore its normal function to the respiratory centre.

ACTION OF CROTON ELLIOTIANUS ON BLOOD

To determine the action of Croton Elliotianus on blood corpuscles, extracts were prepared by macerating the powdered seed in varying quantities of 0.9 per cent solution of sodium chloride. These were placed in the incubator for periods ranging from thirty to forty-eight hours. The fluid was then freed from insoluble matter by filtration and tested on suspensions of mammalian blood corpuscles washed in 0.9 per cent saline solution. In all experiments controls containing the blood suspension only, were set up and subjected to the same conditions as those containing seed extracts. The following table shows the results obtained by addition of different quantities of various percentage strengths of Croton extracts to the blood of certain animals (0 indicates "no haemolysis," + "slight haemolysis," ++ "marked haemolysis," +++ "almost total haemolysis").

Kind of blood	Bullock	Bullock	Bullock	Bullock	Sheep	Sheep	Sheep	Pig	Rabbit
Percentage	1%	1%	2%	2%	1%	1%	1%	2%	2%
Time of observation	23h.	18h.	23h.	24h.	24h.	24h.	42h.	24h.	18h.
Percentage of Croton extract	3.5%	5%	5.2%	10%	9.7%	10%	20%	10%	4%
Percentage mixture with blood 20%	+	++	+	+	+	+	+++	+	+
25%									++
40%	++	total	++	++	++	+++	total	++	
50%									+++
60%	+++	total	total	total	total	total	total	+++	

It is evident from the above table that saline extracts of Croton Elliotianus possess haemolytic activity towards the blood of various animals. This action is however by no means powerful and is slow in occurring; it is seldom and only with strong concentrations that haemolysis is detectable before ten hours but the process is progressive as the following observation shows:

Blood—sheep's corpuscles 1 per cent suspension; Croton seed extract 20 per cent

	TIME OF OBSERVATION		
	18h.	42h.	66h.
20%	+	+++	total
40%	++	total	total
60%	total	total	total
Control.	0	0	0

As evidences seemed to suggest that albuminous matter might be the cause of haemolysis attempts were made to remove this fractionally from the Croton extract. It was found that heating for ten minutes to 80°C. produces a distinct protein coagulum and when this is filtered off further precipitation occurs at 100°C. After removal of each of these precipitates there is a slight diminution of haemolytic activity but this is by no means great. It was inferred that the remaining powerful haemolytic property might be due either to an organic acid or to an acid albumin or proteose uncoagulated after exposure to a temperature of 100°C. Neutralisation of the boiled extracts causes a further albuminous precipitation as does also the addition of alcohol.

In fact by neutralisation of either boiled or unboiled extracts of Croton seeds the haemolytic action can be abolished whilst by the action of alcohol and ether it is appreciably diminished. The latter fact favours the assumption that an albuminous material is the causal agent and this is confirmed by the observation that extracts heated in an autoclave to 150°C. (the sealed tube immersed in air) or in an oil bath at 125°C. lose their haemolytic property although retaining their acid reaction. Superheating of the extract produces a coagulation between the tem-

peratures of 100° and 110°C., the filtered fluid is then found to be free from albumin.

It is a characteristic of several plant proteins to resist coagulation at 100°C. Even ricin in physiological salt solution is not completely coagulated after boiling for half-an-hour.[9]

The following table gives a summary of several experiments conducted on these lines:

TREATMENT	UNTREATED					BOILED FOR 10 MINUTES					TREATED WITH ALCOHOL AND ETHER					HEATED TO 125° C.				
Time in hours	6.5	18	23	42	66	6.5	18	23	42	66	6.5	18	23	42	66	6.5	18	23	42	66
Control....	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20%	0	++	++	+++	total	0	+	+	++	+++	0	0	+	+	+	0	0	0	0	0
40%	0	+++	+++	total		0	++	++	total		0	+	+	+	+	0	0	0	0	0
60%	0	total				0	+++	total			0	+	++	++	++	0	0	0	0	0
40% Neutralized	0	0	0	0	0	0	0	0	0	+	0	0	0	+	+	0	0	0	0	0

It is therefore evident that whilst the coagulable albumins exert some haemolytic influence the larger part of the haemolysis is due to an acid albumin which is precipitated by neutralisation and coagulated by a temperature of about 110°C.

Such results as those just described are obtained with fresh seeds only; when seeds kept for two to three years are employed the results are to some extent inconstant owing probably to decomposition in the albumins.

ALBUMINOUS CONSTITUENTS OF CROTON ELLIOTIANUS SEEDS

A 10 per cent extract of Croton Elliotianus seeds in 5 per cent sodium chloride contains several albuminous substances. Neutralisation of the acid extract with potassium hydrate solution produces a copious precipitation of acid albumin. On removing this, the filtrate when heated undergoes heat coagulation. This coagulum consists of a trace of globulin and a larger amount of albumin, the first precipitated by half saturation and the latter by complete saturation with ammonium sulphate. The

[9] Kobert; Beiträge zur Kenntnis d. vegetab. Hämagglutinine, Berlin, Heft I, p. 5.

clear filtrate obtained after coagulating the albumin and globulin gives a positive biuret reaction and both primary and secondary albumoses can be precipitated by ammonium sulphate. No peptone is present. The seeds therefore contain acid albumin, coagulable eglobulin and albumin, primary and secondary proteoses. Examination of the oil-free powder gave similar results. No reaction for poly-saccharides is obtainable either before or after boiling. The various albuminous bodies give the ordinary reactions for these substances.

In order to determine whether the obvious though slight acidity of 9 per cent saline extracts of the fresh seeds was modified by boiling or superheating a 10 per cent solution of these was prepared and incubated for twenty-four hours. Three portions were taken, No. 1 was filtered, No. 2 filtered, boiled and refiltered, No. 3 boiled, filtered and superheated in an oil bath to 140°C. Of each of the three preparations 1.0 cc. was accurately measured and titurated with $\frac{N}{100}$ KOH.

	per cent
No. 1—16 cc. $\frac{N}{100}$ which equals in terms of tartaric acid	0.120
No. 2—16.2 cc. $\frac{N}{100}$ which equals in terms of tartaric acid	0.1215
No. 3—16.6 cc. $\frac{N}{100}$ which equals in terms of tartaric acid	0.1245

The acidity is approximately the same.

CONCLUSIONS

1. The seed of Croton Elliotianus contains a fixed oil and several albuminous substances.

2. The activity of one seed may differ within narrow limits from that of another derived from the same sample. Seeds kept for eighteen months or two years are apt to be very irregular in their effect, expecially with reference to induction of haemolysis.

3. Towards man the seed is laxative when taken in small dose (0.1 to 0.2 gram) but rapidly purgative (bordering on drastic) in larger amount (0.4 gram).

4. It is also diuretic but this effect, conspicuous after small doses, is masked by the hydrogogue catharsis following larger administrations.

5. Injected hypodermically it occasions haemorrhages both local and remote. It is (in vitro) haemolytic towards the erythrocytes of many animals. Its action in this respect (which is not powerful) appears to be due to acid albumins which show considerable resistance to high temperatures.

6. When introduced into the lumen of the intestine it produces very similar effects to those occasioned by the oil (q.v.) but it is more irritant and its action is rather greater than its oil content alone would produce.

7. No cholagogue effect is attributable to seed or oil but the seed as well as the oil has feebly vermicidal properties towards cestode and nematode parasites.

8. The oil is less irritant than the seeds, its action is much more uniform.

9. It causes no irritation when applied to the human skin nor to the ear of rabbits. Animal experiments show further that intestinal inflammation as a result of local action of the oil is very rarely induced (in these particulars its negative action presents a marked contrast to the positive action of Croton Tiglium oil). Human experiments have in no instance resulted in intestinal inflammatory action.

10. In doses of 0.04 to 0.06 cc. it is a mild laxative and moderate purgative but a vigorous and rapid purgative when 0.1 cc. is administered. Beyond occasionally causing slight nauseation and visceral uneasiness, which may precede but are terminated by purgation, it is peculiarly free from undesirable action.

11. Given inwardly in small dose the oil is feebly diuretic but in man less so than is the seed.

12. Introduction of the oil (or seed) into the lumen rapidly induces movement of the circular wall of the intestine. There is likewise increased secretion from the intestinal glands.

13. Local contractions ensue below and in the neighbourhood of the injection in from ten to fifteen minutes, reach their maximum in twenty-five to thirty-five minutes and then decline, the intestine finally becoming quiescent in about sixty minutes. There are well marked intermissions in spontaneous activity even when the maximum of reaction is present. The large intestine

also responds by contraction to the local stimulation produced by oil or seed.

14. After excitement has ceased a second injection equal in amount to the first is for a time (twenty to thirty minutes) distinctly less effective than the first in eliciting contractions, but if a period of not less than two hours intervenes the results are approximately equal.

15. The local effect caused by such injections into different areas of the small intestine (but especially into the duodenum) predisposes to increased motor response to vagus stimulation which causes contraction, preferentially at or near the site of contact. Response which is usually steady during intervals of spontaneous contraction decreases and may altogether fail when quiescence prevails, but may reappear on repetition of the injection.

16. The latency of vagus response, after commencing stimulation, is usually from seven to ten seconds when the point of injection and sensitive sound are 4 to 5 cm. apart. It appears to be prolonged when these are more distant from one another.

17. A latency of from twenty to thirty seconds is much less frequent but if occurring as a primary phenomenon it may last with slight variation (shortening) throughout the greater part of the observation. It has been noted that the ensuing contractions are frequently more persistant and tonic in character after the longer than they are after the shorter latency. The latter are not hindered by small doses of atropine but large doses usually render vagus excitation inoperative.

18. When the vagus connections with Auerbach's plexus are functionally active, contractions following stimulation appear in the first instance at the site of action of the irritating body within the lumen of the intestine.

19. Intestinal contraction induced by the oil or seed is not arrested by atropine; it may be controlled—though at the height of action very briefly and partially—by stimulation of the peripheral end of the splanchnic nerve. Stimulation of the central end of a mixed nerve (sciatic), both vagi being divided, is more uncertain and always less effective in reducing movement.

20. When the intestine has become quiescent after the excitement occasioned by the introduction of the seed or oil of Croton Elliotianus, renewal of contraction may be induced by the intravascular injection of barium chloride solution or of small doses of nicotine.

21. Injected subcutaneously even when diluted with nine parts of almond oil (both sterile) it does not occasion purgation but a slowly forming circumscribed abscess (free of bacteria) is developed locally.

22. There is no marked febrile action but a well defined reduction of erythrocytes with some leucocytosis occur as temporary conditions. The blood is restored to its original state in eight to ten days.

23. The haemolytic action is a property inherent in the oil which is much feebler in this respect than is the containing seed.

24. Examination of the urine shows that occasionally (once in five observations) blood pigment may appear in the urine with a trace of albumen, coincidentally with the fall in number of coloured corpuscles. This is likewise a temporary phenomenon. The haemolytic action of the oil is much below that of the seed but it is not suspended by neutralisation.

25. The relatively non-irritant action of Croton Elliotianus Oil, its certain effect, whether as a laxative in 8 to 10 hours or in larger dose as a speedy purgative, its high potency in relationship to the small bulk indicate it as a body which would be of considerable value as an addition to purgative remedies for some of the more drastic and irritant of which it would prove a safe and effective substitute.