"INVESTIGATION OF CRATAEGUS OXYACANTHA".

THESIS FOR THE DEGREE OF M.D.

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INTRODUCTION.

Recent events in the political field have had their repercussions on the fields of Science and Medicine. Not least among the changes in the trend of scientific opinion and endeavour originating in considerations of political economy is the post-war tendency to investigate and exploit the possibilities of national self-sufficiency from the point of view of the production of raw materials. The totalitarian state would be independent of all others and the democratic state must needs to some extent follow; from this movement has arisen the recent careful examination of traditional and folk cures in medicine, and of native plants as a source of medicinal virtue.

The Hawthorn (Crataegus oxyacantha) has recently undergone scrutiny in several countries with results which vary somewhat according to the outlook of the investigator.

The publication by Wm. Withering in 1775 of his volume " An Account of the Foxglove etc." is, of course, the classical example of the general revelation of the virtues of an empirical folk-cure. The peculiar virtues of the Hawthorn are of the nature of those of Digitalis; it is not suggested, however, that we have in Crataegus oxyacantha another Digitalis purpurea, but it is proposed that we have

in this plant a medicament of some potency in affecting the cardio-vascular system, and one withal which may well prove to be a useful adjuvant to digitalis itself in special circumstances.

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PART 1.

HISTORICAL.

(a) THE PLANT.

Crataegus oxyacantha derives its name from the extreme hardness of its wood, (craetos = strength), and its thorny form, (oxus = sharp) and spines, (acantha = a prickly plant).

Synonyms: English, Hawthorn; French, aubépine; German, Hagedorn, Weissdorn, Mehlbeeren; Italian, biancospino.

Linnean Class and Order: Icosandria. Pentagynia.

Natural Order: Pomaceae.

Species Characters: The branches are thorny, the leaves smooth, three or four lobed, or five lobed, and serrated. The flowers are corymbose; there are one or two styles. The plant is a shrub or tree which flowers in May or June, exceptionally in April, growing from 5 to 30 feet in height with a typical smooth bark and hard wood. The branches are smooth and thorny; the thorns laterally disposed, awl-shaped and sharp. The leaves alternate, are deciduous and borne on longish slender footstalks; they are smooth or sometimes hairy, deep green in colour, glossy and tapering at the base. The leaf is more or less three-lobed or five-lobed, cut and serrated, wedge-shaped or rounded. The stipules are in pairs, crescent-shaped, cut, deciduous , and vary much in size. The flowers are sweet scented and are produced

in terminal corymbs; they are generally white, but sometimes pink or almost scarlet. The anthers are pink, changing later to black. The styles vary in number, from one to three in different flowers of the same bunch. The fruit is mealy, insipid to taste and of a dark red colour when ripe, but sometimes yellow. The skin of the fruit is tough; the cells are as many as the styles, furrowed externally and very hard.

The above is a standard description of Crataegus oxyacantha, the subject under investigation.

(b) ITS HISTORY IN MEDICINE.

In ancient times the Hawthorn was known though not used as a medicine. Theophrastus of Eresus (371-286 B.C.) in Greece, and Dioscorides of Anazarbus (1 A.D.) in Rome, both describe a thorny bush not unlike Crataegus oxyacantha which was popularly supposed to be the "burning bush" from which God spoke to Moses according to the Jewish tradition. Recent commentaries by Sprengel (1817) and Fraas (1845) would tend to indicate that the descriptions of these classical botanists referred to Crataegus pyracantha, an evergreen shrub of the same family.

Amatus and Marcillus Empiricus in the first decade A.D. mention this same plant of doubtful species as the " crown of thorns " borne by Jesus of Nazareth. There is in the descriptions of early authors a con-

considerable confusion of species, Claudius Galenus (130-201) A.D. undoubtedly describing Berberis vulgaris under the name of oxyacantha. According to Moretum (1601) a commentator of Pliny Senior (A.D. 27-79) the great Roman natural historian described a plant with "spina appendix" which might be Crataegus oxyacantha. It seems to be fairly well established therefore, that Crataegus oxyacantha although mentioned in botanical works in pre-Christian times did not have ascribed to it the medicinal properties of a semi-mystical nature ascribed to some related thorn plants, from their religious associations.

In the works of the"fathers of botany" as one is wont to call the botanists of the sixteenth and seventeenth centuries there are a number of other Greek and Latin plant names which are referred to as Crataegus, mostly very incorrectly.

Thus Bock (1551) , Lonicerus (1543), and Bauhino (1650) refer to it. The first clearly recognisable description of Crataegus oxyacantha was given by the former, called Tragus, in 1551, writing at the township of Hornbach in Germany.

As to when the Hawthorn, until now referred to but little, and that in no way as a plant with medicinal properties, first came to be used as a healing substance, is of necessity unknown. There is little doubt but that its use was gradually developed from the primitive experiments in pharmacology conducted

by herbalists and reputed witches in the countries where it is found. Lonicerus (1543) reports it " officinis inusitata" and Becher (1663) can find no trace of it in apothecaries shops.

The first mention of Crataegus as a medicinal substance in a published work is also obscure, but Bock (1551) gives a recipe for Hawthorn wine, and Becher(1663) also mentions this wine and a tea from the flowers. In the first Swiss pharmacopeia of Hallers (1771) it is mentioned that the housewife prepared a tea from the flowers which was used "in tussi convulsiva aliisque pectoris morbus"; moreover about 1850 this same tea was in use as a household remedy for coughs in Germany and in the Herzegeburg the wine is still used for heart disease. Spielmann's Pharmacopeia (1783) contains a mention of the plant as a source of tanning substances.

Crataegus oxyacantha thus began to appear in the continental pharmacopeiae at the beginning of the nineteenth century - the Spanish pharmacopeia 1803, the French pharmacopeia 1808, Turin 1833, and Gray's supplement to the London pharmacopeia in 1833. Potter (1834) gives a dosage of 10-15 minims of the fluid extract of the fruits four times per day as a "curative remedy for organic and functional heart disorders such as dyspnoea, rapid and feeble heart action, hypertrophy, valvular insufficiency and heart oppression." There is also mention of the dried

powdered fruits in dosage of 2-15 grains.

Virey (1820) mentions Crataegus oxyacantha but purely as a simple for the production of an astringent drink, as also do Esenbeck and Ebermaier (1832). Miquel (1838) gives a short description of the plant and its known empirical uses while Geiger (1829) gives a small note on the bitter properties of the bark and on the use of the fruit as an astringent in diarrhoea. Oken (1841) gives an account of Crataegus oxyacantha as a medicinal plant.

Nevertheless the Pharmacopeia Universalis of 1840 remarks "Die Materia Medica verliert nichts an ihnen". The plant therefore was falling out of official favour in Germany though still used by country people and elsewhere in Europe. Shortly there was to be a recrudescence of attention by the young homeopathic school.

At this time Oken(1843), Karsten (1880) and Merck reported on its clinical advantages in cases of diarrhoea while Schulz (1919) recommends the use of a tea prepared from the leaves in arterio-sclerosis and hyperpiesia. This preparation was known to the Chinese, being described in very similar terms in the herbalist's book Pint Tsa Kang Mu (1562). The species of Crataegus used was Crataegus pinnatifolia. Tunman and Senft (1911) state that a similar preparation is used in Japan.

In 1843 Leroy carried out the first chemical work

on Crataegus oxyacantha, isolating from the bark of the twigs a bitter principle which he termed crataegin. This was followed by Wicke (1851) who stated that the young twigs contained amygdalin but that there was none in the leaves or blossoms. Wicke however referred merely to cyanhydrin, having rather literally interpreted the remarks of Liebeg and Wohler on cyanogens in plants. A further step was taken by Schuchardt (1858) who located propylamine in the flowers. At a subsequent date Greshoff (1908) established the presence of prussic acid in the skins of Haw fruits and reported the occurrence of a fatal case of poisoning by this means. The work of Leroy was repeated by Husemann and Husemann (1871). Wehmer in "Die Pflanzenstoffe" (1911) gives an analysis of crataegus oxyacantha fruits and quotes in addition to those chemical works above mentioned, a few investigations on the ash and salts of the fruits by Peckolt (1910), Kahlbrunner (1851), Perkin and Hummel (1896), and Councler (1870). More recently Brunswick (1923) has stated that there is no amygdalin whatever in Crataegus oxyacantha. An interesting analysis of the whole fruit is given by Konig (1904) and another by Wittmann (1904) while Armstrong (1913) analysed the fruits of Crataegus macracantha. Baechler (1927) gives an analysis of Crataegus oxyacantha and claims to have isolated the active principle, "crataegussäure", a mono-keto acid. This work will be

discussed later.

Subsequent to the loss of interest in the Haw in Germany about the middle of last century, references to the subject are mostly to be found in the publications of French and English authorities.

Gray (1847) gives a recipe for an acidulous drink prepared from the fruits while Guibort (1879) confines himself to a simple account of the plant, while Foursagrives (1885) refers to the fruits as "sennelles" used as an astringent in diarrhoea, the liquid extract of bark used as a febrifuge and to the name oxyacanthine, for some time given to the principle crataegin but later dropped due to confusion with the alkaloid oxyacantha in Berberis vulgaris seeds. The United States Dispensatories of 1902 and 1905 give a slight account of Crataegus oxyacantha while Schelenz (1904) is even more sparing in his reference to it. Tschirch of Leipzig (1909) quotes the work of Greshoff on hydrocyanic acid.

Subsequent to the first American publication on the subject by Jennings (1896) which was of a hyperbolic and unsatisfactory nature an account of some clinical work with Haw appeared in the Kansas City Medical Recorder by Clement (1898). At a later time Huchard (1903), Leclerc (1912, 1919, 1920, 1921) and Renon (1914) gave a series of papers on the action of Crataegus as a clinical hypotensive, with its chief value in cases of arterio-sclerosis with hyper-

tension, taken in small doses over a long period of time. These papers give little detail and no significant figures for comparative purposes.

Reilly (1910) describes the uses of the plant and states that the homeopathic school, while magnifying its value, have used it for its physiological properties and not as a homeopathic drug. He gives a considerable dissertation on its virtues as a safe, non-toxic, non-cumulative, non-diuretic cardiac tonic, which does not raise the blood pressure when given by mouth and is therefore of value in cases of hypertension. Its chief use is as a synergist to digitalis, or given alone with potassium bromide grains 20 as an adjuvant.

Subsequent to Reilly American published work on the subject seems to consist purely of repetitional references in the Standard Dispensatories.

Meanwhile interest in Hawthorn had revived in Germany, several papers being published on "Hagenmark" or Haw marmalade and its chemical analysis. Mezzer and Fuchs (1908), Wittmann (1904), and W.Ludwig (1907) so wrote. This work has been expanded quite recently by Hahn (1933) who reports the presence of vitamin C. in hawthorn berries. Meiling (1937) states that the fresh infusion and the marmalade of Haw contain maximal quantities of vitamin C. They have been used in cases of avitaminosis C.

The extra Pharmacopeia of Martindale and Westcott

(1932) contains reference to the plant as a cardiac tonic, stating that it contains a cyanogenetic glycoside.

In Italy it was discovered by Martini (1932) that the fluid extract of Crataegus oxyacantha was a hypotensive acting on the peripheral blood vessels. This knowledge was put to immediate use by commercial drug houses and in 1935 Simili reported on the use of one of these preparations -"Sedoton", which seems to be similar to the substance"Eurhyton" which also contains crataegus and was reported upon favourably by Silberstein (1927). There are now several preparations which contain Crataegus oxyacantha, bromides, and sundry other plant extracts, marketed as cardiac tonics and hypotensive medicines, even as the drug houses of Paris marketed the stuff under various names at the beginning of the century. It is impossible to tell from the claims of these firms what the clinical properties of Crataegus oxyacantha may be as the proprietary articles contain a sufficiency of recognised and unrecognised medicaments to obtain their effect.

Various preparations of Crataegus oxyacantha have long been used in homeopathic medicine, and extravagant claims for the therapeutic value of this agent are put forward from time to time.

It was introduced to homeopathy in this country by Greene in 1880. For some time the plant was used

empirically and at random until Annulphy of Lizza (1900) reported on it to an International Homeopathic Conference. Schwable (1924) includes it in his book "Homeopathic Medicine." Heinigke (1922) gives an interesting account of toxic symptoms from the exhibition of Crataegus oxyacantha, investigated primarily for the purpose of establishing the homeopathic indications for the drug, since given in detail by Stauffer (1924).

A certain amount of work on this subject has been published in Holland (1898) and elsewhere, but in general the homeopathic literature is scanty and obscure.

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HISTORY OF CARDIAC TONICS OF THE DIGITALIS SERIES.

THEIR ISOLATION AND ANALYSIS.

The cardiac glycosides are a group of naturally occurring substances placed together because of their common characheristic action on the heart. Briefly they may be said to increase the tone, excitability and contractability and the refractory phase of the cardiac muscle, stimulate the vagus centre in the medulla, and slow the rate of conduction through the bundle of His.

It has recently been demonstrated by a variety of workers among whom Armstrong and Armstrong (1931) and Elderfeld (1935) have taken a prominent place, that the cardiac glycosides are related to the sterols and bile acids. The pharacological knowledge of the cardiac glycosides is far ahead of chemical knowledge due to many factors, not least of which is that there are frequently several active constituents responsible for the physiological activity of a given plant extract or pharmacopeal form of the drug. Moreover the active principles occur in very small amounts, are of complex character and usually are labile. They are generally crystalline, bitter and laevo-rotatory, but the crystalline products of attempts at purification are frequently mixtures and the presence of saponins isolated at the same

time makes isolation difficult since solubilities are thereby altered.

The distribution of plants containing cardiac glycosides is wide, the majority of such plants belonging to the Apocyanaceae but also to the Scrophulariaceae, Liliaceae, Ranunculaceae and others. The classical review on this subject of distribution is one by Schmiedeberg (1897), while modern knowledge of the subject has been incorporated in the work of Jacobs (1935).

The foxglove is mentioned in the writings of the Welsh physicians of the thirteenth century, but its action on the heart was best appreciated, not by Withering who used it as a diuretic, but by Cullen (1769) or John Farrier (1799).

Squills is said to have been introduced by Epimenides or Pythagoras but that it was a member of the cardiac glycoside series rather than a diuretic drug was first shown by Fagge and Stevenson (1866), while onage or strophanthus arrow poison from West Africa was examined by Pelikan (1866). Another arrow poison, ouabaio or muju, this time from East Africa was proved by Ringer in 1880 to contain a similar principle, ouabain or g-strophanthin. Canadian hemp was used by Red Indians as a cure for dropsy and Husemann (1876) later showed that "the vegetable lancet" belonged to the digitalis series. In Russia Adonis vernalis is a popular remedy and Bubnow (1883)

allied it to digitalis while Dybkowsky and Pelikan (1861) did the same for ipoh or Antiaris toxicaria, the legendary lethal tree of Malaya. Marmé (1867) analysed Lilly of the valley, Pelikan (1867) Nerium oleander and the same worker in 1868 the fruits of Tanghinia, the ordeal poison of Madagascar. Sassy bark from Senegambia, known as muavi to the natives (erythrophloeum) was added to the series by Gallois and Hardy (1876). Another arrow poison from S.W. Africa echuja, was added to the list by Boehm (1890) on account of its content of Adenium, while Cerbera from India and yozote or Thevetia from Mexico were investigated by Husemann (1876) and Cerna (1879) respectively. Coronilla was examined by Prevost (1896) while Reeb (1898) added wallflower to the list. Three more arrow poisons complete the present survey, Rabelaisia from the Philippine Islands, Calotropis from Lake Tchad, and Pachypodium from Damaraland. These were allied to digitalis by Plugge (1896), Lewin (1913) and Helly (1906) respectively.

With the exception of the toad venoms and the nitrogenous principle of Erythrophloeum guineense the active principles are glycosides consisting entirely of carbon, hydrogen, and oxygen atoms. The general formula may be written thus :- $CH_2(OH).CH.(CHOH)_3.CH.O.R$ (the CH–O–CH forming a ring) On treatment with a hot mineral acid they hydrolyse into a carbohydrate and the aglycone. More or less specific enzymes which can carry out this reaction

are believed to exist in different cells of the various plants which contain the glycosides, separate in life from the glycoside, but acting upon it during maceration of the plant.

The cardiac glycosides from Digitalis and Strophanthus species yield C23 and C24 aglycones or genins which have been investigated by Windaus and Jacobs (1930). These give similar reactions with Tollen's reagent and nitro-prusside and contain an unsaturated lactone group to which their pharmacological properties are ascribed. They also yield the unusual desoxy-methyl-pentoses, digitoxose and digitalose.

Digitalin = Digitaligenin + glucose + Digitalose.

$C_{37}H_{58}O_{14} + 2\ H_2O = C_{24}H_{36}O_5 + C_6H_{12}O_6 + C_7H_{14}O_5$.

Windaus(1924).

Digitoxin = Digitoxigenin + Digitoxose.

$C_{24}H_{66}O_{13} + 3\ H_2O = C_{24}H_{36}O_4 + 3\ C_6H_{12}O_4$.

Windaus (1925).

Digitoxose is $C_6H_{12}O_4$ or $CH_3HCOH.HCOH.HCOH.CH_2.CHO$.

Jacobs (1930).

Digitalose is $C_7H_{14}O_5$ or $CH_3.CH.OH.HCOH.CH.OH.CH.(OCH_3).CHO$.

Killiani (1931).

A. DIGITALIS GLYCOSIDES.

1. DIGITALIS PURPUREA.

Withering's account of the foxglove appeared in 1785 but it was not until 1835 that La Societé de Pharmacie de Paris considered it worth while to offer

a prize to the chemist who would produce the active principle. This challenge was accepted by several, both Nativelle (1869) and Kommolle and Chevenne (1841) naming their product digitaline.

The list of plants at present accepted as giving origin to cardiac glycosides, according to Cushny (1927) is as follows:-

1. Digitalis purpurea.
2. Scilla maritima.
3. Strophanthus kombé etc.
4. Acocanthera Schimperi.
5. Apocynum cannabinum.
6. Adonis vernalis.
7. Antiaris toxicaria.
8. Convallaria majalis.
9. Nerium oleander.
10. Tanghinia venenifera.
11. Erythrophloeum guinense.
12. Helleborus niger.
13. Adenium Bohemianum.
14. Cerbera Odallam.
15. Thevetia iccotli.
16. Coronilla scorpioides.
17. Cheiranthus cheiri.
18. Rabelaisia philippensis.
19. Calotropis procera.
20. Pachypodium scalii.

The cardiac glycosides of definitely determined

structure are given as follows by Elderfeld (1935):-

1. Digitoxin --- --- Leaves of D. purpurea.
2. Gitoxin --- --- --- " " " " .
3. Deacetyldigilanide A. - " " " " .
4. Deacetyldigilanide B. - " " " " .
5. Digilanide A.--- --- Leaves of D. lanata.
6. Digilanide B.--- --- " " " " .
7. Digilanide C.--- --- " " " " .
8. Digoxin --- --- --- " " " " .
9. Cymarin --- --- --- --- Apocynum.
   --- Hydrolysis of Strophanthus glycoside.
10. Allocymarin -- Enzymatic isomerisation of K-Strophanthus -g glycoside.
11. K-Strophanthin -B. --- K-Strophanthus seeds.
12. Periplocymarin -- Periploca graeca wood and bark.
13. Digitalinum verum --- -- Seeds of D. purpurea.
14. Ouabain --- -- Ouabio tree bark.
    -- Seeds of S.gratus.
15. Sarmentocymarin--Seeds of S. sarmentosus.
16. Oleandrin --- --- --- Nerium oleander.
17. Uzarin --- --- --- Wood of uzara.
18. Scillaren A. --- --- Scilla maritima.
19. Proscillaridin A. Enzymatic hydrolysis of Scillaren A.

This list recent as it is has slightly modified the list given by Armstrong in 1931 which may however be quoted as giving in table form an indication of the structure and chemical relationship of these obscure substances.

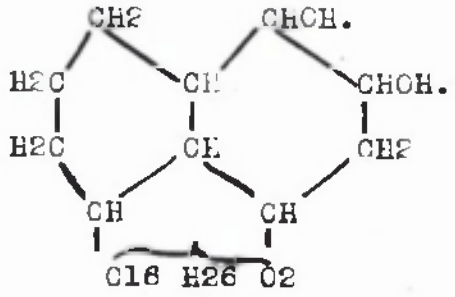
Glycoside	Sugar.	Aglucone.	Formula.
Digitoxin.	3Digitoxose	Digitoxigenin	C23H34O4.
Gitoxin .	Digitoxose	Gitoxigenin	C23H34O5.
Digoxin.	3Digitoxose.	Digoxigenin	C23H35O5.
Lanadigin.	2Digitoxose.	Digoxigenin	C23H35O5.
Digitalin.	Glucose + Digitalose.	Digitaligenin	C23H30 O3.
Oleandrin.	2Digitalose.	Digitaligenin.	C23H30 O3.
Gitalin.	Digitoxose.	anhydro-gitaligenin.	C22H34O5.
Digitonin.	2Glucose + 2Galactose+ 1.d.xylose.	digitogenin	C26H42O5.
Gitonin.	3Galactose+ pentose.	Gitogenin	C26H42O5.
h-Strophanthin-B.	Glucose + cymarose.	Strophanthidin.	
Cymarin.	Cymarose.	Strophanthidin.	
Ouabain.	Rhamnose.	Strophanthidin.	
Periplocin.	Glucose + cymarose.	Periplogenin.	
Periplo-cymarin.	Cymarose	Periplogenin.	
Sarmento-Cymarin.	Glucose + Cymarose.	Sarmentogenin.	
Convalla-marin.	Glucose + Galactose + meth.pentose.	Convallamaretin.	

Glycoside.	Sugar.	Aglucone.	Formula.
Helleborein.	Glucose-Arabinose.	Helleboretin.	
Antiarin.	Rhamnose	Antiarigenin.	

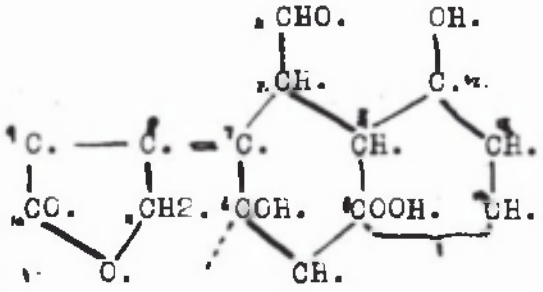
Recently Chen, Anderson and Roberts (1937). have added five additional cardiac glycosides to the list. These are calotropin, a-antiarin, emicymarin, folinerin, and sarmentocymarin.

The details of the molecular structure is known in very few cases, and such as is known is as yet a matter of controversy.

Windaus and Linzert (1925) give gitogenin thus:-

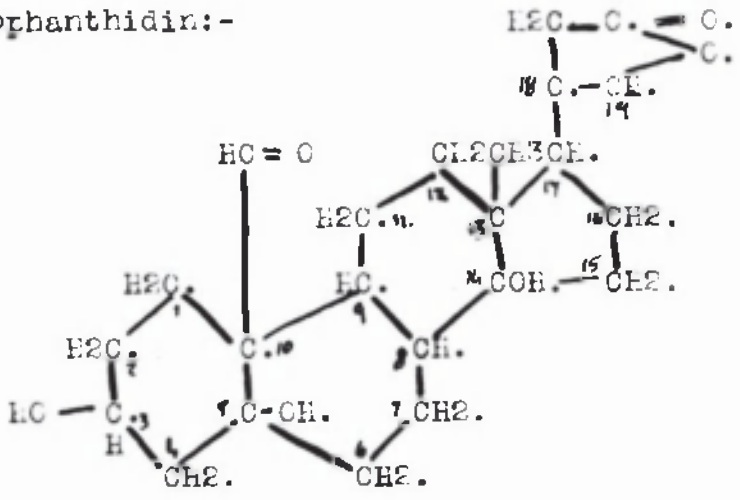


Jacobs and Fleck (1930) give the formula of Strophanthidin as follows:-



Pacheche (1934) gives this new formula for Strophanthidin :-

Strophanthidin:-



Windaus (1928) gives the following list of the glycosides extracted from Digitalis purpurea and their chemical relationship :-

C41H64O13 + 3 H2O = C23H34O4 + 3 C6H12O4.

Digitoxin Digitoxigenin Digitoxose.

C41H64O14 + 3 H2O = C23H34O5 + 3 C6H12O4.

Gitoxin Gitoxigenin Digitoxose.

C35H56O12 + 2 H2O = C23H36O6 + 2 C6H12O4.

Gitalin Gitoxigeninhydrate Digitoxose.

C36H56O14 + 2 H2O = C23H34O5 + C6H12O6 + C7H14O5.

Digitalinum verum Gitoxigenin Glucose Digitalose.

Saponin.

C35H90O29 + 5 H2O = C26H42O5 + 4 C6H12O6 + C5H10O5.

Digitonin Digitogenin Hexose Pentose.

8. DIGITALIS LANATA.

Digitalis lanata glycosides are closely related to and in many cases identical with those of D. purpurea. The principle work on this series has

been done by Smith (1930) and Stoll and Kreis (1933).

B. APOCYANACEAE.

1. STROPHANTHIDINS.

There are forty odd species of the Strophanthus family containing cardiac glycosides and there is considerable confusion between them as to their exact content of glycosides. In 1868 Arnaud first discovered ouabain in the roots and bark of the ouabaio tree, a species of Acocanthera used as an arrow poison by the Somalis of East Africa. Ouabain is a substance which possesses an unusually strong power of crystallisation, thus rendering its isolation and purification comparatively simple. For this reason it has been used as a standard in the assay of commercial Digitalis and Strophanthus preparations. A classical review of strophanthidins is contained in the volume by Fraser of Edinburgh (1891).

2. NERIUM OLEANDER.

This member of the family Apocyanaceae is a plant the poisonous properties of which were known in antiquity, Hippocrates having recorded observations thereon.

3. THE COYOTE.

The medicinal properties of the coyote plant were known to the Aztecs. Herrara (1876) showed that it contained a cardiac glycoside thevetosin.

C. ASILEPIADACEIAE. -- Periplocin.

D. LILIACEAE.

Scilla maratima, the origin of Squills, and Convallaria majalis belong to this family.

E. RANUNCULACEAE.

Adonis vernalis has been known since the times of Hieronymus Tragus and Hellebore since Hippocrates. Franzen (1931) has isolated three substances from the latter with digitaloid characteristics. These are Cellianin C21 H35 NO2; Sprintillamin C28 H45 NO4; and Sprintillin C25 H41 NO3.

The aglycones are the characteristic portion of a glycoside. The structures of strophanthidin, periplogenin, digitoxigenin and gitoxigenin have been determined with a reasonable degree of accuracy. A beginning has been made on the study of scillaridin A and the toad venoms. The structure of uzarigenin is known except for the positions occupied by two of the hydroxyl groups. The structures of the remaining members are at present unknown, although it is probably safe to predict that , in general, they will be found to conform to the same pattern as those which have been studied in detail.

The intensity of the physiological activity of the glycosides appears to depend to a large extent on the presence of a lactone grouping in the aglycone. This lactone consists of a side chain of four carbon atoms which constitutes the most characteristic portion of the molecule. The double bond is found

in this side chain. Many of the typical reactions of the aglycones, such as the production of a red colour with alkaline nitro-prusside solution (Legal's Test), reduction of Tollen's reagent, and the presence of an "active" hydrogen as shown by the Zerewitinoff Test, have been shown to be conditioned by the presence of this unsaturated lactone side chain. These properties are all characteristic of $\Delta^{\beta\gamma}$ unsaturated lactones as shown by Jacobs and Hoffmann (1926). The nature of this side chain has been shown to be that of a lactone of an enolised γ-aldehydo acid attached by its β carbon atom to the main tetra-cyclic system. Jacobs and Elderfeld (1935) degraded a derivative of digitoxigenin to a saturated acid which proved to be identical with etiocholanic acid. This proved that the carbon skeleton of the cardiac aglycones is identical with that of the sterols and bile acids and that the lactone side chain of the former is a fragment of the side chain of the latter and is attached to the skeleton at the same point ie. at carbon atom 17.

NITROGENOUS CARDIAC PRINCIPLES.

Any account of the digitaloid substances would be incomplete without mention of another group of very interesting compounds which are, however, of animal origin - the so called toad venoms. These substances, which exhibit a potent digitalis action, are secreted by the parotid glands onto the

surface of the animals. Abel and Macht (1911) were the first to report the isolation of a non-nitrogenous, crystalline principle, bufagin, from such a secretion of the tropical toad bufo agna. In addition a relatively large amount of adrenalin was isolated. The chemistry of these materials has been recently developed to a considerable extent. As in the case of the plant glycosides confusion has been occasioned by uncertainty as to the source of the material under examination. This has particularly been the case in the study of Ch'an Su or Senso (the dried venom of a Chinese toad). Wieland and Weil (1913) isolated a substance bufotalin from the venom of bufo vulgaris which corresponded to but was not identical with bufagin. Subsequently Wieland and Allee [1922] obtained a non-glycosidal, neutral, nitrogenous substance bufotoxin, which was shown to be a conjugation of bufotalin with argenine and suberic acid. Thus it was concluded that bufotalin is a sort of "genin" of the toad poison in question, bufotoxin, in which the suberylargenine parallels somewhat the position of the sugar in the non-nitrogenous glycosides.

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HISTORY OF CARDIAC TONICS OF THE DIGITALIS SERIES.

THEIR ASSAY.

The method of biological assay in the preparation of standard potencies of a drug is a twentieth century development of Materia Medica. It is obvious that study of the effects of the cardiac series of drugs could not be made until the particular preparation being used in the clinique or laboratory could be accurately compared with other similar preparations as to potency. As preparations can not be compared at random with one another the first step is to chose an empirical standard preparation, preferably recognised internationally.

For Digitalis " the Standard Preparation for Great Britain and Northern Ireland is a mixture of dried and powdered digitalis leaves, kept in sealed vials in the National Institute for Medical Research, Hampstead, London. The Standard Preparation for other parts of the British Empire is the same, except for those countries in which a similar standard preparation, kept in a different institute, has been defined by law; in these countries the Standard Preparation , so defined, is used": so declares the British Pharmacopeia of 1932.

The proposal to prepare an International Standard was put forward at a conference in Edinburgh

in 1923 by Magnus of Utrecht who subsequently prepared and lodged the Standard at Hampstead. This standard was a mixture of ten different samples of digitalis leaf, powdered, dried at 55-60°C and sealed in ampoules. Since the mixture was made from ten different samples, its activity was presumed to be about equal to the mean activity of samples of digitalis: the standard was, therefore, available for any one to compare with it an unknown sample of leaf to see how far this sample differed in activity from the mean activity of digitalis leaf.

In 1935 the Standard prepared by Magnus was nearly exhausted and the Permanent Commission on Biological Standardisation set up by the League of Nations Health Section decided to adopt the British Preparation of 1935 to be known as the International Standard Powdered Digitalis (1936), lodged at Hampstead. This consists of nine different samples of the leaves of Digitalis purpurea obtained from four British and American growers, which had been dried at 55-60°C. These samples of powder were dried for twenty four hours at 50-55°C after mixing and distributed to ampoules in a warm dry room. The filled ampoules were kept for six days in a low pressure chamber over calcium chloride at 30-35°C before being sealed; the final water content was found to be about 3%. The British Standard thus prepared was compared in various laboratories

with the International Standard of 1926 by the frog method and the cat method. Whereas the potency by the frog method was 1.4 times that of the 1926 Standard, the potency by the cat method was 1.6 times (Burn 1937). The National Institute for Medical Research, London recommends that the new Standard be regarded as 1.25 times as active as the old, without respect to the method of comparison applied.

An International Digitalis Unit is defined as being the equivalent in potency of 0.1 gm. of the International Powder. Thus one unit of activity previously (1926) contained in 0.1 gm. of Standard Powder is now (1936) contained in 0.08 gm. of Standard Powder.

Edmunds, Moyer and Shaw (1937) give a table of comparisons which is interesting :-

%potency of Standard Powders in terms of International Standard as 100%.

Method.	U.S.P.	British.	Canadian.
1 hr. frog.	134.22%.	118.87%.	115.77%.
4 hr. frog.	157.37%.	132.50%.	124.70%.
12 hr. frog.	191.40%.	143.00%.	125.40%.
Cat.	141.65%.	121.6%.	119.26%.

There are a considerable number of methods of biological assay applicable to powdered digitalis and tincture of digitalis, making use of the frog, cat, guinea pig, pigeon, dog, goldfish and even mice.

The methods most commonly used, and the ones followed in this investigation, are the cat method of Hatcher and Brody (1910) and the frog method as used by Trevan (1926). There are also chemical methods of assay.

A. THE FROG METHOD.

This method was introduced by Houghton in 1898. In it an injection is made under the skin of the frog and it is left for 1 hr., 3 hrs., or overnight as preferred. This technique is carried out on a suitable number of frogs, which are then killed and the heart exposed. Those in which it has stopped in systole are counted as dead, those in which it still beats are counted as survivors. The 1 hr. method is official in the U.S.A. Pharmacopeia but the 12 hr. method permits of a sharper distinction between survivors and dead. If digitalis leaves are being assayed a tincture is made from them, if tincture is used the alcohol is driven off and the volume restored with water or saline. It is necessary to use frogs of the same sex in a test, taken between October and May, as during the breeding season the sensitivity of a frog to cardiac tonics varies. The doses are adjusted according to body - weight and groups of frogs of similar weights must be used for the Standard and the unknown tincture.

The details of the technique are given in the experimental section of the present thesis.

Trevan has calculated that for this method the expected standard deviation if 48 frogs be used is 10%. Chapman and Morrell (1931) obtained less deviation in the characteristic curve they built up on Canadian frogs with ouabain. Among other frog methods advocated is that of Focke (1913) which takes into consideration the time between the injection and systolic arrest of the heart, and gives the result in "valors".

$$\text{Valor} = \frac{\text{weight}}{\text{dose by time.}}$$

Schmiedeberg (1910) advocated perfusion of the frog heart and Watanabe (1923) suspension of the frog's auricle. These methods have been entirely superseded by Trevan's injection method based on the work of Houghton.

B. THE CAT METHOD.

Perfusion of the isolated mammalian heart as suggested by Haynes (1906), and the injection of intact white mice or guinea pigs has been superseded by the technique of Hatcher and Brody (1910) as modified by Mijngaarden (1926). The details of the technique are given later but essentially it consists of slow intra venous infusion under constant pressure of a saline dilution of the tincture of digitalis, or of infusion of digitalis , to a cat under general anaesthesia with ether, and with artificial respiration via a tracheotomy tube from a respiratory pump. The lethal dose is measured directly.

Wijngaarden (1926) states that the average error using one animal is 13%. If enough animals are used for the mean deviation to be less than 6.67 /n-1 then the error is 6.6%. On an average four cats are sufficient and it is not necessary to compare with a standard each time. Tincture of digitalis may keep its strength unaltered for one year.

The chief point of controversy about the cat method arises from the fact that the average lethal dose of a sample of digitalis leaf is smaller the greater the amount of ether used. Further it was shown by MacDonald and Sclapp (1930) that the variation in figures for different cats tested with a given sample was much greater when ether was used than when the digitalis was administered to spinal cats. Many papers have been written on the subject of which the following is a short summary :-

Rowe (1919) and Chopra and Chowan (1932) recommend intra-peritoneal chlorbutanol; Epstein (1933) used intra-peritoneal paraldehyde 1.6 to 1.8 gm. per kilo in a 10% solution; Fulton, Liddell and Rioch (1928) used urethane 1.8 gm. per kilo intra-muecularly; Haag (1927) advocates the use of spinal cats prepared according to the technique of Pollock and Davies (1923), while Macdonald (1934) and David and Rajamanickam (1934) surveyed a variety of anaesthetics and showed that variation decreases in

order for ether, paraldehyde, chloretone, chloralose and urethane, and that the choice of anaesthetic affects the lethal dose largely.

The end point is best determined by recording the carotid blood pressure and noting its terminal sharp fall.

C. GUINEA PIG METHOD.

This method, introduced by Knaffl-Lenz (1926) and practised by Gage (1933) consists of the slow intra venous infusion of modified tincture of digitalis into the jugular vein of a medium -sized guinea pig anaesthetised with subcutaneous urethane and a little ether; the heart is directly observed at the end of the experiment.

D. THE PIGEON METHOD.

This method depends upon the fact that digitalis given intravenously to pigeons causes emesis; it was introduced by Hanzlik (1929). The modified tincture is given in small doses (0.1 cc. per 100 gm.) into the wing vein by a small bore needle. The birds may be used again in a few days for comparison of the standard and unknown.

F. OTHER METHODS.

Esveld (1937) describes a method of assay of digitalis given by mouth in the decerebrate cat. Pittenger (1919) suggested the use of goldfish where the solution of digitalis may be added to the water, but it is doubtfull whether the action here

noted is primarily on the heart or the gills.

Schneider (1925) used Paramecia as an experimental animal for assay purposes, observing the movement by microscope, while Viehofer and Tubis (1929) used Daphnia.

The variety of methods suggested reveals the innate difficulties of, and objections to the biological method with its large number of uncontrollable variates in arriving at a precise figure for the potency of a drug. Essentially the method is a comparitive one between the toxicity of a standard and the unknown preparation.

Finally there have been developed a number of methods for assaying the active principles of digitalis by chemical means.

Among these the method of Knudson and Dresbach (1923) has had most success, but even that has not in any way replaced the biological method. Martindale (1912) introduced a method depending on the coloimetric reaction of the active constituents of digitalis with Froehde's reagent - sulphuro-ammonium molybdate. It only estimates the approximate activity and takes three hours to complete. Beery (1919) modified this method. It was not shown by either of these investigators that the colour reaction varies in accordance with the physiological activity of the glucosides. Baljet (1918) introduced a method using picric acid in alcohol

which overcomes that difficulty and is very sensitive. The method of Knudson and Dresbach mentioned above is similar th Baljet's method. Lendle and Schmelzer (1935) declare that only Scillaren does not give the colour reaction.

de Lind van Wijngaarden (1927) gives the following figures comparing the biological method with the colorimetric method: the colorimetric method is not used in practical standardisation work but is of theoretical interest.

Dig.Fol.	Cat Method.		Colorimetric Method.	
Standard	Value in mg.	Relative value.	cat value in mg.	Relative value.
1923.	91.	100.	71.	100.
A.	156.	58.	102.	69.5
B.	80.8	112.6	59.	120.

HISTORY OF CARDIAC TONICS OF THE DIGITALIS SERIES.

THEIR PHARMACOLOGY.

The action of digitalis and drugs of the type known as "digitalis - like" was first examined on the frog heart and was so typical for all the cardiac glycosides as to be considered characteristic. Vulpian in 1855 initiated a long series of researches on the subject which ultimately established the following points, though not without controversy and misunderstanding amongst various schools of thought.

The exposed frog heart may be observed, or attached to a recording apparatus, or excised and perfused. Whatever the method employed it is noted that on administration of the minimal lethal dose of the drug no change may occur in the heart beat for some time or slowing occurs with increase in the size of the beat, systole being more complete and diastole sometimes more marked. Under Strophanthus Santesson (1897) remarked an acceleration in the rate of contraction in 25% of frogs. Occasional intermissions in the ventricle are followed by ventricular half rhythm, then the establishment of auricular half rhythm, ventricular quarter rhythm and cessation of the ventricle in diastole. Eventually both auricles and ventricles cease to

act and diastolic arrest is established. The ventricle may pass into a state of partial or complete contracture, especially if stimulated from without.

Eismayer and Quincke (1929) give the following illuminating table of time relations in the frog heart under strophanthus.

Strophanthus.	Amplitude.	Duration.	Quotient.	Duration of Syst.	Diast.
Before.	47 mm.	1.6 sec.	3.4	0.9	0.7
5 min. after.	47 mm.	1.45 "	3.1	0.78	0.67
15 " " .	42 mm.	1.20 "	2.9	0.6	0.6
25 " " .	18 mm.	1.0 "	5.6	0.38	0.62
30 " " .	14 mm.	0.98 "	7.0	0.38	0.6

If the heart is observed under a moderate dose of digitalis early slowing is frequently absent and complete emptying of the ventricle with the appearance of a bloodless condition of the chamber on systole is noted, the ventricle relaxes less in diastole, the period of contraction is lengthened, and finally a phase of "peristaltic action" with dissociated contraction and relaxation of different portions of the ventricular wall is seen. Half rhythm is established at the beginning of this phase and if the dose be lethal the ventricle is arrested in systole, while the auricles, having failed to force blood into the contracted ventricle, are arrested in diastole and contrast with the white chamber below. Recovery from such a dosage is

commoner in strophanthus poisoning than in digitalis poisoning. Large intavenous doses cause an immediate passage from normal rhythm to contracture but after arrest of the beat the ventricle reacts to external stimuli, showing that the arrest is partly due to blockage of the normal stimulus to the ventricle from the auricle via the conducting system.

The change in rhythm and change in tone which comprise the action on the frog's heart are due to direct action on the muscle, systolic contracture arising from the effect of digitalis upon the general contractile tissue, and diastolic arrest by the action of digitalis upon the bundle of His by slowing the rate of conduction. Jacobj (1900) states that small doses of the digitaloids have an apparent depressant action on the frog heart when perfused through it and often cause diastolic arrest. In the early stages of poisoning the auricles and ventricles are synchronous.

Loewe and Wichels (1920) suggested that the presence of ganglia was essential to the specific digitalis action on the heart, while Amsler and Fick (1920) refuted this.

The "negative dromotropic effect" of digitalis is its most marked feature, but as to whether in the early phases under a minimum dosage there is a "positive dromotropic effect" as stated by Schonleber (1920) is doubtfull. The rate of emmission of

impulses from the pace maker is often unchanged, or is slower, more rarely quicker; the sinus venosus survives the rest of the heart. The conduction of the impulse is generally impaired in the A-V fibres and later in the S-A fibres.

In the first stage of action the excitability of the ventricle is greater than before, but soon it is found that an electric shock which is sufficient to cause a contraction in the normal heart when given during diastole fails to cause any response during the action of antiarin and the other glucosides (Sollmann 1914). Nevertheless there is evidence to show that the tendency of the ventricle to develop a spontaneous rhythm is increased as is shown by Schmiedeberg's experiment (1875). The contracture which culminates in the arrest in systole is essentially in its beginning an imperfect relaxation; in the stage of peristalsis the ventricle may become acid to litmus. The efficiency of the frog's heart under digitalis varies according to its previous state of nourishment.

There is always a latent period between the time when a cardiac glycoside reaches the heart and the time of development of its action, but the action once begun is only reversible in the early stages as shown by Issikutz (1915). Straub (1922) found that scillaren was more easily washed out of a perfused heart than strophanthidin, as also are

antiarin, cymarin and digitalein. There is doubt as to whether or not the cardiac glycosides are fixed in the heart cell or simply diffuse into it. De Giacomi (1926) reports that digitoxin is irreversible, digitoxigenin reversible, gitalin and bigitalin partially reversible. This is supported by Lenz (1926) and Fischer (1928) but contradicted by Rothlin (1927) and Kingisepp (1935). The matter is thus in doubt.

In the mammalian heart there are somewhat similar changes . The effect of digitalis on the ventricle is compounded of two factors, augmented inhibition through the vagi and the greater strength of the systolic contraction. The first slows the rhythm and often increases the relaxation and lowers the diastolic pressure; the second increases the extent of the contraction and the systolic pressure, and tends to limit the extent of relaxation and the fall in diastolic pressure. In the auricles the same factors are in action. The conducting fibres (A-V bundle) are at first but little affected; later conductivity is greatly diminished. The work of the heart is, in this early phase, gratly increased; larger doses cause excessive inhibition and the efficiency falls, though the output per beat is increased. The rhythm becomes irregular, that of the auricle very slow, that of the ventricle sometimes more frequent than the auricle from idio-

ventricular beats.

According to Pick (1924) the excised bundle of His beats with its own rhythm and reacts to digitalis in a manner essentially similar to the whole heart.

Partial block of the A-V bundle causes intermissions of the ventricle. According to Ackermann (1873) the slow pulse of digitalis is due to the increasing activity of the inhibitory mechanism, and is entirely removed by atropine, the action of digitalis occurring chiefly at the vagus centre in the medulla, though some degree of peripheral action occurs also. Etienne (1909) states that the slowing may be removed entirely by section of the vagi in normal animals. The effect of central stimulation is due to reflexes from the heart. The isolated perfused heart therefore does not show this slowing in the first stage and the lethal dose is higher than for the heart in situ. Obden and Nielsen (1938) report that digitalis causes no sensitisation of the pressor or chemo receptors of the carotid sinus and the cardio inhibitory effect is presumably due solely to a sensitisation of the heart muscle to acetyl choline. Strophanthin and acetyl choline are synergic in effect on the break down rate of phospho-creatine. The action is thus peripheral rather than central.

The changes in contractility are due to direct action on the muscle fibres of both auricle and

ventricle. The power of the muscle is increased though Bock (1898) denies that the absolute strength is augmented under helleborein. Bodo (1928) working on the heart-lung preparation showed that strophanthin causes a diminution of coronary outflow and a rise in blood pressure. This has been confirmed on the Langendorff preparation by Mancke (1929).

The electrocardiogram shows an inversion of the T wave with lengthening of the P-R interval following upon large doses of digitalis. Further exhibition of the digitalis-like glycosides leads to toxicity marked by a great increase in the tendency to spontaneous beats in different parts of the heart which may lead to the development of ectopic rhythms and ends in fibrillation, and a rapidly increasing difficulty in conduction of impulses between auricle and ventricle. These conditions arise from changes in the heart muscle itself, being seen in the excised and perfused heart.

The hypodynamic perfused mammalian heart resembles the frog heart in responding to the digitalis series with an increase in the amount of work done increase in the strength of contraction, contracture and impairment of conduction, while the spontaneity is not augmented.

Large doses of digitalis given intravenously cause a moderate rise of blood pressure with a slow

heart and increased size of pulse wave, later followed by increase of pulse rate and a further marked rise of pressure. The first recording of these effects was made by Blake (1939). A part of this effect is due to increased cardiac output in the early stages of administration but mostly it is due to vaso-constriction. All members of the digitalis series perfused at constant pressure through surviving arteries constrict them and so reduce the rate of flow. Similar constriction occurs if a ring of artery be suspended in a solution of the glycoside, but in the case of the coronary artery dilatation is observed (Cow 1911). The majority of investigators state that in poisonous doses they constrict the coronary arteries, which are more sensitive to them than are the systemic vessels, while in therapeutic doses there is little or no effect. The limb vessels may dilate under strophanthin but contract under digitoxin (Gottlieb and Magnus 1902) as do those of the brain. The general blood flow is accelerated by digitalis.

Strophanthin is not so powerful in its effect as digitaline: the action in the latter case is a direct one on the vessel wall, in the former case there is a slight degree of action through increased power of vaso-constrictor impulses in the nerves (Sollmann and Filcher 1914). There is however no evidence that the blood pressure or the vessel walls are affected

by therapeutic doses of the glycosides.

Injected hypodermically the glucosides generally cause pain and discomfort, and digitoxin in particular, which is insoluble in the body fluids, gives rise to inflammation, in the dog. Their general action is that of local irritants to the skin and mucous membranes, and local anaesthetics if the action is prolonged. The taste is bitter. Large doses cause a feeling of nausea and faintness with vomiting, and later colic and diarrhoea. Small haemorrhages may occurr in the bowel wall. This action has been shown by Hatcher and Eggleston (1912) to be central and is used as an indicator in the method of biological assay using pigeons. With the nausea occur s sweating and increased salivation.

Digitalis and its allies tend to increase the pulmonary ventilation in animals, especially when given in large doses, but the respiration can be arrested by a concentration which does not arrest the heart. These effects vary somewhat from animal to animal. Diuresis occurs under squills, digitalis, apocynum etc. but is best marked in cases with a dropsy of cardiac origin. Solids are increased in amount but to a less extent than fluid, giving a pale, neutral urine. As there is no evidence of a concomitant state of renal vaso-dilation it is believed that diuresis follows upon general improvement of the circulation with hydraemia.

Diuresis can be induced in hutch rabbits. Cushny and Lambie (1921) state that moderate doses of digitalis have no diuretic effect on normal animals and that large doses tend to be anti-diuretic.

The medulla oblongata is stimulated by digitalis and in the white rat, which is resistant to the cardiac action of digitalis, this may extend to the lower brain, giving rise to a condition resembling strychnine poisoning (Hatcher 1912). Antiarin has a marked effect.

Greene, Boutwell and Peeler (1915) state that strips of isolated turtle ventricle are augmented and accelerated by digitalis even after atropine.

The spinal chord of the frog is depressed and in the mammal muscular weakness of central origin is present during digitalis poisoning. Digitalis is not an anti- pyretic of any importance: it causes myosis when given in large doses. A distinct reduction in the activity of the muscles and nerve endings in the frog in prolonged digitalis poisoning is seen. This is a direct action analagous to the action on the heart (Neuschlosz 1922).

Unstriated muscle is acted upon by lower concentrations than are needed to affect striated fibres. Unstriated muscle is thrown into a state of increased tone by the cardiac glycosides and with larger doses some forms of it relax. The action is a direct one, unaffected by atropine. The bronchi

also constrict; in so far as examined these actions take place in the intact animal.

According to Trendelenburg (1913) digitalis has no effect on excised bronchial muscle. Gross (1914) states that large doses of digitalis given intravenously by ear to the intact rabbit depress the respiration while smaller doses cause a preliminary stimulation, while with the same technique Santesson and Strindberg (1916) report direct effects on the medulla oblongata as shown by dyspnoea, myosis, debility, paralysis, tremors, convulsions, and loss of reflexes.

Annelids are little affected by digitalis but *medusae enter* a state of contracture of the hood and tentacles.

Digitalis has certain characteristic effects on the electo-cardiograph which are essentially the same for all animals. de Boer (1919) showed how digitalis affected the electro-cardiograph of the frog heart, shortening the R-T interval, lengthening the Q-R interval, and inverting the T wave; while with larger doses a curious effect of alternating complexes of positive and negative form was obtained. This was confirmed by Laubry and Deglaude (1924). Pietrkowski (1917) had obtained similar results on the isolated frog heart. On the heart of intact dogs Lewis, using strophanthin (1921) found a similar lengthening of the P-R interval, unaffected by vago-

tomy or atropine, with inversion of the T wave. This was confirmed on the isolated heart by Sakai (1918). Levine and Cunningham (1917) give the following interesting results:-

Inversion of the T wave by 25%
First extrasystole by 40%
Idioventricular contraction by 70%
Dissociation of the ventricles 80%
} of the L.D.in cats, given intra-venously.

while Hoekstra and Schleussing (1933) give :-

Inversion of the T wave by 57%
Absence of P wave by 65%
} L.D. strophanthin i.v.

In man the electo-cardiograph has been studied clinically for long and the effects of digitalis are well known. These effects are similar, P-R lengthened, and T inverted with therapeutic doses. Cohn and Levy (1920) and White and Sattler (1916) showed this some time ago, while more toxic stages with heart block were recorded by Brams and Gabermann (1931).

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HISTORY OF CARDIAC TONICS OF THE DIGITALIS SERIES.

THEIR THERAPEUTIC APPLICATIONS.

During the nineteenth century very little progress was made with the use of digitalis in disease though its use became widespread, and squills and strophanthus became of recognised value. Apocynum, convallaria and other members of the series failed to establish themselves in this country except in cases of individual preference. The work of Mackenzie and later of Wenkebach (1901) developed the study of cardiac function and ellucidated the common irregularities of the heart to be met with in clinical medicine. From this time the action of digitalis and its therapeutic uses came to be better understood. Ultimately the special action of digitalis in auricular fibrillation was appreciated and the fallacy concerning the contra-indication of the drug in aortic disease exploded.

With regard to administration of the drug, many members of the series have not been used in therapeutics and little comparison of their effects has been tried. The action on the heart under digitalis, squills, and strophanthus is the same, but the former is more likely to cause vomiting and the latter two to cause diarrhoea; helleborein in doses of three grains per day had no effect on the heart and

apocynum had very temporary effects, according to Whiting (1918). The dosage of digitalis made use of varies in different hands, and in cases where there is no urgency the method made use of by Withering and formulated by Mackenzie, of employing the cumulative action of the drug to establish its effects safely is preferred. Eggleston (1920) advocated the elimination of the latent period inevitable in this method of treatment, by the exhibition of a massive dose according to the patient's weight followed by lesser doses for purposes of maintenance.

Various modifications of this method have been made since eg. that by Pardee (1920) and Fraser (1922). For such intensive treatment the tincture employed must be assayed. According to Jacobs (1933) and Hamilton (1918) the tincture of digitalis loses 10% of its strength in one year. There seems to be no advantage in giving digitalis other than by mouth; strophanthin may be given intravenously. The leaf is potent in tablet or pill form; the infusion is little used though Weiss and Hatcher (1921) found that a carefully bottled infusion kept its strength over two years. When rendered alkaline the infusion rapidly loses its virtue (Watanabe 1923). Tincture of strophanthus is but slowly and imperfectly absorbed from the alimentary canal and its use has fallen somewhat in abeyance in the face of a flood of widely advertised commercial preparations of

digitalis. Strophanthin grain 1/240 intravenously is a standard method of using the drug in an emergency. The use of cymarin is in the experimental stage, but apocynum tincture is well known: its effects are evanescent and it tends to cause nausea and vomiting early. According to Mavin and White (1921) convallaria has similar drawbacks. Squills has only one third the strength of action of digitalis in tincture form, and tends to cause diarrhoea. Helleborein and erythrophloein are inferior in action.

The preparations officially recommended in the British Pharmacopeia (1932) are as follows:-

1. Digitalis Pulverata. Dose 1/2 - 1 1/2 gr. 3-10 gr.
2. Infusum Digitalis Recens. m90-300. oz. 1-4.
3. Tinctura Digitalis. m 5-15. m30- 90.
4. Strophanthinum. 1/240 - 1/ 60 gr.
5. Tinctura Strophanthi. m 2-5.
6. Scilla. 1-3 gr.
7. Oxymel Scillae. m 30-60.
8. Syrupus Scillae. m 30-60.
9. Tinctura Scillae. m 5-30.
10. Acetum Scillae. m 10-30.

None of the other members of this series or their preparations are official.

With regard to the therapeutic actions of digitalis, this series of drugs is indicated in auricular fibrillation with ventricular tachycardia, in auricular flutter, in either case whether congestive

heart failure is present or not: and in cogestive heart failure, especially if the amount of urine is diminished or dropsy is present, whatever the cardiac lesion may be.

Auricular fibrillation in man was first described by Cushny and Edmunds (1907) though long known as pulsus irregularis perpetuus. If a case of auricular fibrillation with rapid irregular ventricular action be treated with digitalis marked slowing of the pulse occurs, the efficiency of the circulation rises, and the pulse becomes more regular until at a rate of 40 to 50 per minute it may be full, powerful and quite regular. The signs of auricular activity are not restored and the electro-cardiograph shows the characteristic tremor of auricular fibrillation. Subjective symptoms improve, and the area of cardiac dullness is often reduced in size. The effect is best seen in recent cases of auricular fibrillation following upon a rheumatic lesion of the mitral valve, and may be little seen in long standing cases with pronounced arterio-sclerotic change , though diuresis may occur and be of benefit: little benefit accrues if the auricular fibrillation be unaccompanied by ventricular tachycardia.

In many cases, compensation having been restored by digitalis the drug may be discontinued for months

The rate of fibrillation in the auricle is frequently increased due to augmented activity of the

vagus (Lewis 1922), while increasing A-V block in the bundle of His excludes this augmentation in the rate of auricular impulses until idioventricular rhythm is established. This inhibitory effect may be at first effected by means of the central stimulation of the vagal inhibitory mechanism, but is soon a direct effect on the A-V bundle (Cushny 1927). The block by digitalis in auricular fibrillation is favoured by malnutrition of the heart and fatigue of the A-V bundle from the too rapid passage of stimuli. If the action of digitalis be pushed a new irregularity of the pulse, coupled beats or pulsus bigeminus, may develop. The electro-cardiograph shows this to consist of a normal systole followed closely by a ventricular extra-systole of unknown origin, probably associated with the tendency of digitalis to set up increased spontaneity in the ventricle. More serious indications of overdosage are runs of extrasystoles, multi-focal extrasystoles or ventricular tachycardia. Undue slowing of the pulse may occur in cases of auricular fibrillation due to undue depression of conductivity in the bundle of His, in normal rhythm to depression of the sinus, when ventricular escape may occur.

In auricular flutter digitalis exerts its effect by increasing the degree of A-V block already present; in a proportion of cases flutter is changed to auricular fibrillation which, on discontinuation of the drug

may give way to normal rhythm: thus its action may be curative as well as ameliorative, whereas in auricular fibrillation of spontaneous origin it is never curative. The increase in the A-V block is due to increase in vagal inhibition which, according to Lewis (1920) is also responsible for the change from flutter to fibrillation.

In partial heart-block digitalis is contra-indicated as tending to increase the number of intermissions, but in total or complete heart block it may be of value in strengthening ventricular systole; the presence of extra systoles is no indication for the exhibition of digitalis. Pulsus alternans may or may not be improved by it. In many degenerative conditions with normal rhythm subjective improvement occurs under digitalis which may also be attended by diuresis. Hypertension, arterial disease and aortic incompetence are not contra-indications for the use of digitalis if other symptoms and signs suggest its employment. Cardiac asthma is frequently relieved. Where there is pyrexia or thyreo-toxicosis digitalis fails to slow the pulse. Its use in diphtheria and pneumonia is a matter of controversy.

With regard to diphtheria Edmunds (1937), McCullough (1921), Bush (1919) and Haskell (1927) were of opinion that diphtheria toxin and digitalis were synergistic in action and that consequently the drug is contra-indicated in diphtheria.

Dieckloffe and Shulze (1937) recommend the use of strophanthin in small doses to increase the endurance of the heart weakened by diphtheria toxins. It also increases the central inhibition of post rheumatic tachycardia with normal rhythm in children, where the action is on the pace maker. Acute endocarditis is a contra-indication, unlike the state of ventricular hypertrophy which is often present in decompensated hearts which improve under digitalis. According to Cohn and Fraser (1919) digitalis is of value in cases of mitral stenosis due to the lengthening of the interval of conduction in the A-V bundle permitting of better emptying of the auricle, while Christian (1919) shows that aortic regurgitation is no contra-indication to the use of digitalis. (long before this)

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HISTORY OF CARDIAC TONICS OF THE DIGITALIS SERIES.

THEIR TOXICITY.

The effects of digitalis tend to be cumulative and to outlast the administration for a considerable time. Thus toxic effects are not unknown, and indeed Withering in his classical treatise on the subject declares ""Let the medicine therefore be given in the doses, and at the intervals mentioned above; let it be continued until it either acts on the kidneys, the stomach, the pulse or the bowels; let it be stopped on the first appearance of any one of these effects and I will maintain that the patient will not suffer from its exhibition , nor the practitioner be disappointed in any reasonable expectation".

Here we see that the toxic effects on stomach and bowel were recommended to be used as a control of dosage, much in the nature of a "biological assay" of each patient. Indeed this method is quite frequently used in practice today. Toxic doses produce vomiting, cardiac irregularity and sudden heart failure.

As was seen in the review of the pharmacology of the digitalis aries of drugs the toxic phase of action on the heart appears late in the administration of the drug or with large doses. In the frog irregularity of the ventricle passes into a state of "plastic tone" or contracture: sino-auricular or auriculo

ventricular block may occur while if the sino-aricular junction be crushed as in the first Stannius experiment the ventricle does not come to a standstill but develops at once its spontaneous rhythm.

Digitalis is absorbed and stored in the heart muscle, remaining there for a considerable time in active form; a similar effect occurs in mammals. In the intact mammal according to Hirschfelder (1920) hyperpyrexia increases the toxicity of digitalis considerably. In toads the effect of digitalis is diminished and leads to diastolic rather than systolic arrest of the heart. In the perfused mammalian heart the ventricles assume a very rapid rhythm and a systolic tendency, finally passing into fibrillation and ceasing in systole. The effects on the intact heart may best be divided into a vagal or inhibitory stage and a muscular stage characterised respectively by a slow rhythm, diastolic tendency and lessened conductivity, and by increased tone, heightened irritability, ventricular spontaneous rhythm and extra-systoles. Independence of the auricular and ventricular contractions results in curious periodic variations in the volume of the pulse, the rhythm at this time being fairly regular. At a later stage extra-systoles destroy the normal rhythm and the pulse is intermittent. Rodents, toads and grass snakes are very resistant to digitaloids, while the rat has a much higher lethal dose than the cat or the rabbit. Frogs

and guinea-pigs have seasonal variations in their resistance.

In the clinical use of digitalis the full therapeutic effect practically coincides with the minor toxic manifestations, but according to Robinson and Wilson (1918) the therapeutic dose is only one third of the lethal dose. The toxic phenomena may be grouped under digestive disturbances and minor phenomena; cardiac irregularities; and fatalities.

The digestive disturbances occur early and subside quickly if the drug be stopped. Headache, malaise nausea, and vomiting are common, while diarrhoea occurs more frequently with hellebrein, squill and strophanthus than digitalis. The emetic action is not local or central but results from the effect of reflexes from the heart, Hatcher and Weiss (1923). Gastric motility and secretion are increased, and the emptying time reduced by twenty percent, as shown by Liere and Sleeth (1938). Digitalin is the most emetic in action of the glycosidic principles.

A few cases of exanthemata of an urticarial nature and of yellow vision and nystagmus have been reported.

The cardiac irregularities may take many forms, the most common being that of excessive vagus stimulation (sinus irregularity). Ventricular extrasystoles are common. The next stage is that of partial heart block, which may be permanent and occur

with relatively small doses, while with very large doses extra-systoles, coupled beats, increased blood pressure and pulsus alternans may be found - Sollmann (1936).

According to Levine (1919) fifty percent of the lethal dose produces toxic irregularities in the heart of the cat; thus in the human severe toxic effects may result from less than twice the therapeutic dosage.

The effects of chronic poisoning with digitalis in animals varies somewhat according to reports. Lhota (1910 and 1912) studied the effects of continued administration of digitalis in dogs and rabbits and Klein (1914) in cats. They report the typical cumulative toxic effects, with some evidence of the acquirement of tolerance in rabbits and dogs. There has been much study on the matter of accumulation of digitalis with the attendant risk of toxic effects. Meynert (1937) divides the digitaloid glycosides into three groups of which the first (digitoxigenin, digilanide B) are the first to exert their action and the most rapidly detoxicated; the second group (digitoxin, digilanide A) have a slower action; while the third group, comprising the rest of the digitalis glycosides acts most slowly and tends to accumulate in the heart, vessels and liver.

Van der Heide (1885) gives a fine review of Hegevand's work on the effect of chronic digitalis

poisoning on the histology of various organs. Hatcher (1912) describes how the rat shows almost exclusively nervous symptoms under such treatment, while Fraenkel (1903) studied cumulation of hellebrein in rabbits. Lhota (1910) abandoned guinea pigs as being very susceptible to digitalis but this has not been confirmed since. On cats he performed experiments resembling the "combined ouabain technique" of Hatcher. North and Spung (1937) give a detailed account of the effect of chronic digitalis poisoning on the electro-cardiogram and histology of the heart muscle in cats. With repeated administration of high doses of digitoxin-Merck they obtained firstly a flattening and inversion of the T wave of the electro-cardiogram , and later the T wave coming off high in the S portion of the complex (infarct type). The animals were killed and histological investigation revealed necrosis of the papillary muscles and sub-endocardium of the inter-ventricular septum.

Loewit (1914) reports that there are no morphological changes in the healthy or diseased heart after prolonged treatment with any of the series. It has been suggested that cymarin reduces the number of oxidase particles in the rabbit and guinea pig heart. Impens (1910) denies this finding.

Fatalities are uncommon if the drug be given by mouth in fractionated doses as emesis and other toxic symptoms set in first and lead to the dis -

continuation of the drug. Single large doses may have a fatal result, which is accompanied by the signs and symptoms indicated above and frequently results from a paroxysm of dyspnoea. The termination is apt to be delayed for several days after administration. 2.5 grams of digitalis folium has been fatal while 4 grams has been attended with recovery.

The intravenous administration of fatal doses of strophanthin into cats produces vomiting, diarrhoea and convulsions, (Hatcher 1909). In man intravenous medication has not infrequently been attended by fatal effects, when the accumulation of previous digitalis medication has been underestimated; death is probably due to ventricular fibrillation, as shown by Levine (1919).

The introduction of the methods of biological assay has resulted in the accurate determination of the minimum fatal dose of various of the digitaloid series on several animals. As these doses give an idea of the relative potency of the drugs in question they are given below:-

Potency as lethal doses in mgm. drug per gm. frog.

Crude drugs.		Principles		Commercial Prep
Digitalis	1.5	Strophanthin-K	0.001	Digitalin-
Squill	1.2	Strophanthin-M	0.00094	German 0.05
Strophanthus	0.0075	Ouabain	0.00048	
Convallaria	0.25			

By the one-hour frog method the minimum systolic doses in mgm. per gm. frog are:-

Digitalis	0.5-1	Digitoxin	0.0085	Digipuratum	0.(
Strophanthus	0.0075	Strophanthin-M	0.0011	Digitalin-G	0.0'
		Ouabain	0.0003	DigitalinFr	.0
			0.001	Digitalein	.02

With the cat method of Hatcher and Brody these results are given in mgm. of drug per cat unit.

Crude drugs.		Principles.		Commercial Preps.	
Squill	575	Adonidin	4.35	Digitalin G.	3.6
Euonymus	475	Digitalein	3.55	Nativelle	0.8
Helleborus	100	Convallamarin	1.7	Ouabain Arnaud	
Digitalis	100	Helleborein	1.7		0.06
Apocynum	70	Digitalin v.	1.5		
Convallaria	50	Scillitoxin	0.4		
Stroph. k.	3.0	Digitoxin	0.3		
Str.hispidus	1.5	Strophanthin-M	0.13		
		Ouabain	0.1		

Recent results by the 12 hr. frog method in mgms. drug per gm. frog are as follows:-

Digitalin	0.0061	g. strophanthin	0.0008
Digitalein	0.005	Antiarin	0.0016
Gitalin	0.006	Coronillin	0.005
Digitoxin	0.0036	Oleandrin	0.0022
k strophanthin	0.00075		
Cymarin	0.0008		

The following two tables are given by Hatcher and Haag and Woodley (1934) respectively:-

Glycoside.	Dog.	Cat.
Digitoxin	3.27 mgm/K	0.4 mgm/K
Strophanthin	.16-.18 mgm/K	0.1 mgm/K

i.v. L.D.

	Cat.	Pigeon.
Ouabain	0.089	0.195
Strophanthin	0.088	0.235
Digitoxin	0.318	0.376

L.D. in mgm. per K.

Again Rothlin (1934) gives this list :-

Digitaline	0.0105	mgm./	gm.	heart	weight.
Digilanide	0.0093	"	"	"	"
Digoxin	0.009	"	"	"	"
Ouabain	.0019	"	"	"	"
Scillaren A	.0032	"	"	"	"
Scillaren B	0.0024	"	"	"	"

The whole matter is summarised very excellently by the table given by Lendle (1935). This makes it easy to note at a glance the relative toxicity of the digitaloid glycosides. This table is given on the following two pages:-

Substance.	Frog.	Mouse.	Rat.	Guinea Pig.	Rabbit.
	Lymph sac.	Subcut.	Subct.	Subct.	Oral.
Adonidin.	4.0	-	-	-	-
Adonis.l.	2.5	-	-	3-4	170
Acocantherin.	0.5	-	-	5-7	23
Antiarin.	6-16		-	-	-
Convallamarin.	5-15	600	-	40	320
Convallarin.	0.7	70	-	5	2000
Coronillin.	5	-	-	2	-
Cymarin.	0.7-2	-	-	-	-
Digitalin.	6-22	-	120	5-16	20
Digitalein.	5-32	11-65	-	-	-
Digitoxin.	3-6	14	-	-	100
Erythrophloein.	20	-	-	-	-
Gitalin.	4-6	29	-	-	-
Gitoxin.	8.5	-	-	-	-
Helleborein.	-	-	-	-	-
Neriin.	20-50	95	-	-	-
Oleandrin.	2.25	-	-	-	-
Periplocin.	25.	-	320	-	-
Scillaren.	.8-1.1	-	10	-	0.9
g.Strophanthin.	.4-1	8-15	50	.1-.3	8-20
h.Strophanthin.	-	-	-	0.2	-
K.strophanthin.	.75-1	5-19	60	0.4	80

Rabbit.		Cat.			Dog.	
Subct.	I.V.	Oral.	Subct.	I.V.	Subct.	I.V.
-	5	-	-	3	-	-
7-30	1-25	-	-	0.7	-	0.5
-	0.25	-	-	-	-	-
-	1	-	-	-	-	-
6-40	6-40	-	-	1.7	-	-
1.5	1.5	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	0.5	-	-	-
15	3	-	4	-	-	-
-	-	-	-	.125	-	.215
-	1-6	0.25	.35	3.35	-	-
-	-	-	-	0.3	0.5	-
-	5.8	0.37	0.55	0.53	-	-
-	-	0.88	0.8	0.59	-	-
-	-	-	-	1.9	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	0.85
10	-	-	2.5	-	-	-
0.7	0.45	-	-	-	-	-
.1-.4	.1-.2	-	.15-.2	.1	.1	.125
.125	.2-.4	-	-	-	-	-
0.55	.25	-	0.3	0.3	0.13	0.11

PART 11.

PRESENCE OF A DIGITALIS-LIKE EFFECT FROM LIQUID EXTRACT AND TINCTURE OF CRATAEGUS OXYACANTHA.

CHEMISTRY.

The present investigation of the properties of Crataegus oxyacantha consisted of a preliminary investigation to determine whether or not the fruits of this plant contain some substance of a digitaloid nature. This having been established attention was next turned to the chemistry of the substance or substances and an endeavour made to extract the active principle. At the same time the chemistry of the material was investigated along various lines, and the work of Baechler (1927) repeated.

In the autumn of 1937 there was a particularly heavy crop of Hawthorn fruits and a large collection of these was made, after the species of the plant had been determined, and the fruits taken to the laboratory in November 1937. There they were spread out in the drying room until thoroughly dried, then cleaned of twigs and leaves and stored in a dry cool place.

Working on this material and on the supposition that the material contained an active principle with the action of a cardiac "digitaloid" drug which was capable of extraction, the work of Baechler was repeated, in so far as it seemed of significance.

Baechler's Method.

250 gms. of powdered dry whole Haw fruits are placed in a Wester's extractive apparatus (a form of reflux condenser) with 750 cc. petroleum ether and extracted continuously until no further extractive comes over. This is removed and the extraction repeated with methylated ether. The ether extract is evaporated to dryness and the residue dissolved in warm acetone of a minimal volume and then placed in the refrigerator for twenty four hours; it is then centrifuged. The precipitate is taken up in warm acetone, refrigerated and re-centrifuged. A grey precipitate is obtained: this substance is "crataegusaure" or crataegic acid. Baechler claims that this substance is a pure principle, and is the specific active principle of Crataegus oxyacantha.

The continuous extraction apparatus of de Wester (1913) was slightly modified by the use of a shielded electric bulb placed in apposition to the reservoir as a heating apparatus.(see diagram). This provision was taken to avoid the danger of fire with the extremely volatile and inflammable fluid in use.

Protocol. 21/7/38.

250 gm. powdered whole Haw fruit plus 500cc. petroleum ether extracted for 12 days until no residue was left on evaporating some of the fluid.
Extractive equals 7 gms. of a green, greasy solid.
Extractive removed and fluid dried from off the

Haws. Extraction repeated with methylated ether until no further extraction occurs, after 21 days.
Ether collected and evaporated.
Ether soluble fraction equals 3.45 gm.
Dissolved in 200 cc. warm acetone and put in the refrigerator at 4°C for 24 hours in a corked 500 cc. flask.
22/1/38.
Acetone centrifuged; a brownish precipitate of .43 gm. weight obtained. Dissolved in 20 cc. warm acetone and replaced in refrigerator for 24 hours.
23/1/38.
Centrifuged. Grey precipitate of .2 gm. weight. Non-soluble in water or alcohol, soluble in ether, chloroform and acetone: non-crystalline, greasy, not reacting with HCl or ammonia.

This substance is the crataegic acid of Baechler. His assertion that the substance isolated is the specific active principle of Haw is based on a short series of experiments in which the isolated heart of the frog (R.esculenta) was perfused according to the Straub technique with a five per cent emulsion of crataegic acid in gum acacia. The effects produced by this treatment were variable and consisted mainly of a diminution in the systolic excursion of the lever in the apparatus, without loss of diastolic tone, and occasionally of slowing and of augmentation of the beats. As such effects could

manifestly be obtained by perfusing a frog heart with gum acacia solution, and as the substance is water and alcohol insoluble its specificity was suspect.

Accordingly, there being no reasonable way of testing the crataegic acid obtained by perfusion or by direct application to the frog heart, the following experiments were performed:-

1. Four frogs, male, weight 25 gm.
Injected into the lymph sac .25 cc. alcohol containing in suspension circa .02 gm. crataegusaure.
Examined after 1 hr., 4 hrs., and 12 hrs. All survived.

2. Four frogs, male, 26.5 gm.
Injected into the lymph sac .5 cc. saline containing .02 gm. crataegusaure in suspension.
Examined after 1 hr., 4 hrs., and 12 hrs.; all survived.

3. One male frog, weight 25 gm.
Received .04 gm. crataegic acid implanted under the skin of the thigh, the incision being sown up and left.
This frog was examined as before. It survived.

Accordingly, if a dosage of this substance in the nature of .16 gm. subcutaneously per 100 gm. frog fails to prove lethal, while a dose of .0061 gm. digitalin, .0022 gm. oleandrin, .005 gm. coronillin, or .0008 gm. cymarin given by lymph sac is lethal

Diagram No 1.

Westen's Apparatus

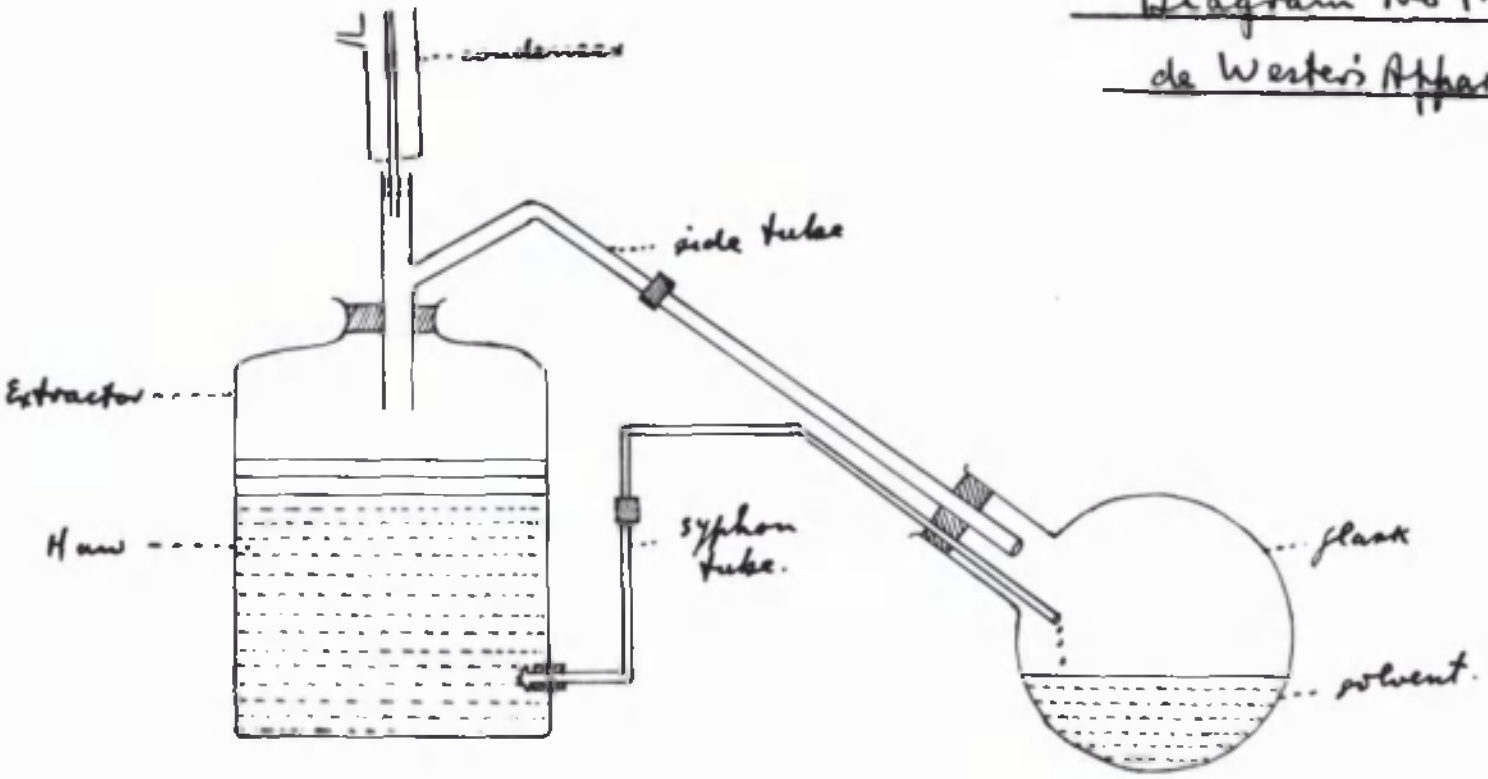

Diagram No 1.
de Westen's Apparatus.

then this substance is not of a comparable nature. As further work abundantly proves that the fruits of Crataegus oxyacantha contain a principle of the "digitaloid" type, it is concluded that the substance isolated by Baechler in the manner described above, and shown by him to be an acid of empirical formula C31 H51 O2 COOH, is not specific.

Having therefore concluded that the substance produced by Baechler and declared by him to be specific was not so, it remained to endeavour to isolate that substance. Accordingly the general extractive method of Rosenthaler (1904 and 1920) was employed.

General Investigation.

(a) Micro-Sublimation.

The micro-sublimator of Klein (Rosenthaler 1920) consists of a strong glass boiling tube containing a few grams of the substance to be tested, in powder form. Over this, inside the tube, is fitted a circular glass coverslip with a fine hole bored in its centre. The tube is corked and the cork admits a fine piece of glass tubing packed in glass wool and fitted against the central hole in the cover glass with a seal of vaseline. (see diagram). This piece of glass tubing is attached to a suction pump which is set a-running and the whole tube heated in a sand bath. After a variable time the apparatus is allowed to cool and the cover glass removed and examined under the micro-

scope. The sublimate may then be tested chemically.

5 gm. of powdered dry whole Haw fruit was put in the micro-sublimator and heated for two hours, cooled and the cover glass examined under a low power of the microscope. This revealed a quantity of yellow fluid material and a number of crystals of various shapes:-

(a) colourless feathery crystals

(b) colourless prisms and rhomboids.

The surface of the cover glass was then tested chemically as follows, a fresh glass having been prepared for each test.

1.With two drops of 10% KOH.

A red colour resulted, denoting the presence of methyl-oxy-anthraquinones. (Rosenthaler).

2.With two drops of Iodine in KI.

A precipitate would have denoted the presence of certain alkaloids. There was no precipitate.

3.With two drops of KHgI 2.

A precipitae would have denoted the presence of certain alkaloids. There was no precipitate.

(b) Water Extraction.

10 gm. of powdered dry whole Haw fruits was warmed to 55°C with water in a beaker, cooled, filtered and the filtrate taken and tested as follows:-

1.With litmus paper. The rection was acid.

This may have been due to acids, acid salts, tannins

or phenols.

2. 5 cc. filtrate with 2 cc. 5% ferric chloride soln. A dark colour resulted.

This reaction denotes the presence of tannins.

3. 20 cc. filtrate plus 10 cc. saturated solution of neutral lead acetate. This gave a heavy cloudy precipitate.

This denotes the presence of acids, tannins, mucins and proteins.

Basic lead acetate solution was added until the solution turned alkaline to litmus paper.

A further precipitate of a mucilagenous and flocculent nature resulted, denoting the presence of gum arabic or glucosides.

4. 5 cc. filtrate plus 2 cc. freshly prepared Fehling's solution boiled for one minute.

A precipitate denotes the presence of glucose. There was no precipitate.

6. 5 cc. filtrate plus .5 cc. HCl boiled for five minutes, cooled and neutralised with NaOH. 10%.
5 cc. of this solution plus 2 cc. freshly prepared Fehling's solution boiled for one minute.

A red precipitate resulted, denoting the presence of disacharides and/or glucosides.

(c) Acid Extraction.

5 gm. powdered dry whole fruit of Haw plus 15 cc. 1% HCl warmed to 50°C for five minutes, cooled and

filtered.

A drop of the filtrate is put on a slide with a drop of (a) Iodine in KI (b) KHgI 2.

The absence of a precipitate denotes the absence of an alkaloid.

There was no precipitate.

(d) Alkaline Extraction.

5 gm. powdered dry whole fruit of Haw plus 20 cc. water plus 3 gm. sodium bicarbonate shaken up thoroughly.

A persistent colourless froth which was not removed by boiling the solution denoted the presence of , saponins.

(e) Picrate.

Picrate paper was prepared by soaking strips of filter paper in a solution composed of one per cent picric acid solution 10cc. plus 10 cc. saturated solution of sodium carbonate, and drying.

1. 1 gm. powdered dry whole fruit of Haw plus 5 cc. water plus .25 cc. N/1 sulphuric acid in a flask with a piece of picric paper between the cork and the neck of the flask.

2. 1 gm. powdered dry whole fruit of Haw plus 5 cc. water plus .5 cc. chloroform in a flask with picrate paper.

3.1 gm. powdered dry whole fruit of Haw plus 5 cc. water plus .5 cc. chloroform plus a pinch of emulsin

in a flask with picrate paper.

After twenty four hours the flasks were examined and it was noted that the colour of the papers was unchanged.

Flasks numbers (2) and (3) were heated on a water bath and number (1) boiled with the cork loose. The paper remained unchanged in colour. There was therefore no cyanogenetic glucoside present eg. amygdalin.

Thus the original contention of Wicke (1854) is again refuted.

The preliminary work having tended to indicate that there were no glycosides present in the Haw fruit, extraction was begun in the Wester's apparatus according to the method of Rosenthaler.

STEP A.

250 gm. powdered dry whole fruit of Haw plus one litre of petroleum ether extracted by continuous process for twenty days until no further residue was left on a watchglass on evaporating a little of the extracting fluid. The extracting fluid was then collected and evaporated at a low temperature on the electric hot plate until 50 cc. of a pale yellow fluid remained. This was shaken three times for five minutes with a total volume of 150 cc. distilled water containing three drops of HCl; this extraction was performed in a separator funnel and the fluids separated.

The acidulated water was now alkalinised to litmus paper with ammonia solution, and shaken with 50 cc. of petroleum ether.

The petroleum ether was separated and evaporated.

The residue weighed 0.4360 gm.

The whole was taken up in 10 cc. of saline and one cc. of this injected into the lymph sac of a frog of 27.5 gm. weight. The frog survived.

5cc. were then injected. The frog was inspected at intervals of 1 hr., 4 hrs., and 12 hrs. It survived.

It was concluded that no specific principle had been isolated by this process.

STEP B.

Process A.

The petroleum ether from the first separation from acidulated water was shaken with 150 cc. distilled water to free it from acid, separated, and evaporated off. 6.7 gm. of a thick oily residue were left. This was taken up in 200 cc. boiling alcohol and decanted. The residue was fat and oil of 3.4 gm. weight.

This substance, of 3.4 gm. weight was of a pale, creamy appearance and hard consistence, with a greasy feel. It melted on heating, to an oil, floated in water in which it was insoluble, as also in dilute and absolute methyl alcohol. It left a greasy stain on paper, and on heating with dilute NaOH saponification occurred and the process could be carried by heating

with dilute sulphuric acid to the production of a fatty acid with a most peculiar odour, and which left a greasy stain on paper. On shaking with water the soap dissolved and created a foam.

The residue was thus a fat, or a mixture of fats and oils.

Process B.

The 200 cc. boiling methyl alcohol was cooled rapidly and filtered, which left on the paper a large amount (3 gm.) of yellow waxy material with oily drops among it. The drops were ignored and the waxy material tested as follows:-

1. .5 gm. material dissolved in 5cc. chloroform plus three drops conc. sulphuric acid gave a violet colour in the sulphuric acid and a brown colour in the chloroform - ?Hesse's reaction.

2.1 cc. water plus 5 cc. sulphuric acid plus a portion of the material gave no colour reaction. 1 cc. iodine in KI turned chocolate and then black.

Moleschott's colour reaction negative.

3. .5 gm. material dissolved in 5 cc. hot glacial acetic acid, cooled, and a few drops of sulphuric acid added. On cooling a white flocculent precipitate appeared, the colour remaining unchanged on addition of the acid.

Liebermann's reaction negative.

4. .5 gm. material plus two cc. conc. HCl plus .5 gm. solid FeCl3 evaporated to dryness on a watch glass

gave a brilliant red and blue colour.
Mach's reaction positive.

The material was thus shown to consist of waxes and sterols of no glycosidic activity.

The method of extraction advocated by Rosenthaler having yielded no specific principle from Haw, using petroleum and methylated ether as extracting agents it was determined to tackle the problem from the point of view of the infusion or watery extract which was known to be active. Accordingly the following methods were tried:-

(a) 1 kilo of powdered dry whole fruits of Haw were infused for fifteen minutes in a porcelain pot with three stirrings, with five litres of boiling distilled water and filtered hot through glass wool. After cooling, one litre of saturated solution of basic lead acetate was added and the whole left for twenty four hours, and then filtered in a Buchner filter. 500 cc. saturated solution of basic lead acetate were added and left for one hour, and the whole filtered through double Watman's No.1 paper. The filtrate was a clear fluid. Hydrogen sulphide from a freshly prepared Kipp's apparatus was passed for four hours and the whole filtered on a Buchner filter, and re-filtered. A clear colourless fluid resulted. This was evaporated to dryness in open porcelain dishes over steam during which time the colour deepened from lemon to yellow and finally

resulted in 173 gm. of a tarry substance which could not be further dried. This mass was thoroughly extracted by stirring with ether, chloroform, and methyl alcohol in that order and these fluids evaporated.

(a) The ether extract was an amorphous substance of a pale yellow colour, weight .43 gm.

It was taken up in 10 cc. saline and, on the first day 1cc., on the second day 5cc. injected into the lymph sacs of a frog of 37 gm. weight. The frog survived.

(b) The chloroform extract was a yellow amorphous substance of 1.2 gm. weight.

It was taken up in 10 cc. saline and 5cc. injected into the lymph sacs of a frog of 24 gm. weight. The frog survived.

(c) The alcohol extract was a dark amorphous substance of .73 gm. weight.

It was taken up in 10 cc. of saline and 5 cc. injected into the lymph sacs of a frog of 28 gm. weight.

The frog survived.

It was concluded that by these methods no specific active principle had been isolated from Crataegus oxyacantha.

(b) The Method of Schnitker and Levine.

250 gm. powdered dry whole fruits of Haw was infused for 15 minutes in a porcelain pot with 500cc.

boiling distilled water and filtered hot. This was acidified with glacial acetic acid to a pH of 2 using 8cc. as a test sample and methyl red (alcoholic solution) as an indicator.

To this was added an equal volume of 95% alcohol and the whole heated to 50°C on a water bath, and filtered hot. This was placed in an open porcelain dish and put in a drying room with an electric fan directed upon it until the volume was reduced to 15cc. To this was added 150 cc. 95% alcohol and the whole dried on a water bath at 40-45°C, resulting in 5.1 gm. of a red gummy substance. This was dissolved in 20cc. 10% alcohol and 15 drops of ammonia added until it was just alkaline to litmus, which resulted in a muddy blue precipitate. This was extracted three times in all with 90 cc. chloroform in a separator funnel and the chloroform evaporated on a hot plate.

The residue was clear and gummy in nature, weighed 1.7 gm. and was taken up with 3 cc. chloroform, 10cc. ether, and 71 cc. of 90% benzol, whem an opalescent fluid resulted. This was left for 48 hrs. in the refrigerator to separate out. It was then decanted and centrifuged when .1 gm. greyish white powder resulted.

This was taken up in 10cc. saline and 5cc. injected into the lymph sace of a frog of 29 gm. weight. The frog survived.

The 20 cc. 10% alcohol rendered alkaline and

extracted with chloroform was diluted to 100 cc. with Ringer's solution and a frog heart perfused with it through Greene's cannula. The fluid proved to be rather toxic to the heart but did not have a specific action.

It was concluded that no specific principle had been isolated by this method from Crataegus oxyacantha.

(c) The following method was adopted.

200 gm. powdered dry whole fruit of Haw infused for 15 minutes with one litre of boiling distilled water and filtered on a Buchner filter. Tri-chlor acetic acid was added until on testing a centrifuged specimen with further addition of acid, no further precipitate came down. The whole was filtered twice and evaporated at 40°C to dryness. This yielded 15 gm. of a brown gummy material, which was extracted thoroughly with ether to remove the excess of acid, and then taken up in 250 cc. of Ringer's solution. This was tested on a frog heart by the perfusion method of Greene when it had a toxic effect, which however differed in several particulars from that of simple infusion of Crataegus oxyacantha, and was admirably duplicated by .1% solution of tri-chlor acetic acid.

It was therefore concluded that the effect had been due to the presence of residual traces of acid, and that the method was unsuitable for the extraction of

any substance which must subsequently be tested biologically. It was therefore abandoned.

(d) The work to date having proved unsuccessful it was concluded that the specific substance was not capable of being extracted from Raw by ether, alcohol or chloroform but only by water; it was further concluded that the methods used for preparing a protein-free filtrate were of such a nature as to inevitably destroy the specific principle, or remove it during the process.

Accordingly the method of Stewart and Chatterji (1937) was employed.

250 gm. powdered dry whole fruit of Raw was infused for 15 minutes with 500 cc. boiling distilled water and filtered. 250 cc. 1% HCl was added and the whole filtered on a Buchner filter after 10 minutes.

250 gm. kaolin was washed on a Buchner filter with alcohol, chloroform and ether and dried, then added to the previous filtrate and shaken vigorously on a shaker for one hour. This was filtered on a Buchner filter, washed once with water and decanted into a mortar. This was ground with 10% aqueous solution of sodium carbonate in minimal quantities until alkaline, when it turned a faint blue colour. It was then ground with anhydrous sodium sulphate until it was sticky and left in an evacuated aspirator over soda lime until it dried solid. The result was

a white powdery mass.

This was extracted for 12 hours in a large Soxhlet apparatus with chloroform, when a pale yellow fluid was obtained which was evaporated to 25 cc. on a hot plate and left for 24 hours in the refrigerator at 4°C to crystallise out. The yield was .1182 gm. of a brown amorphous powder sparingly soluble in Ringer's solution. This was tested biologically by perfusion through a frog heart with Greene's cannula, when it had a weak specific action in a dilution of .01%.

The taste was bitter and lasting, as of an acidic substance.

Protocols. 8/12/37.

Above repeated. Yield of .0850 gm.

Active but not strong in a dilution of .001%.

5/1/38.

Repeated using methyl ether as the elutor of the kaolin. Yield of .020 gm.

Inactive.

7/1/38.

Repeated using 97% alcohol as the elutor of the kaolin. Yield .120 gm.

Active but very weak at a concentration of 1%.

It was concluded that the active principle was water soluble and could be removed therefrom by kaolin from which it was best eluted by chloroform, but that the loss of potency as a result of this process was such as to render it impractable.

(e) Accordingly an attempt was made by the method of Bernthsen (1931).

50 gm. powdered dry whole fruit of Haw was infused in a porcelain pot for 15 minutes with 500 cc. boiling distilled water containing 50 cc. N/10 sulphuric acid, and filtered. 100 cc. of the filtrate was taken and neutralised with N/10 ammonia and centrifuged. The precipitate was taken up in 10 cc. N/10 sulphuric acid and again neutralised carefully. 50 cc. Ringer's solution was added and this perfused through a frog heart.

There was no effect other than might have been attributed to the inorganic ions.

The method was abandoned.

(f) The method of obtaining a protein-free filtrate recommended by Eggleton and Eggleton (1938) was next applied.

100 gm. powdered dry whole fruit of Haw was infused in a porcelain pot for 15 minutes with 500 cc. of boiling distilled water, and filtered. 400 cc. were rendered isotonic with Ringer's solution and used in experiments on perfusion of the frog heart by Greene's cannula. 100 cc. of infusion were taken and 50 gm. anhydrous sodium sulphate added, left for 15 minutes, filtered and kept for 24 hours at 4°C in the refrigerator. A mass of $Na_2SO_4.10H_2O$ with a precipitate had come down after that time, and was

filtered off, leaving as filtrate a 4% solution of sodium sulphate. This was dialysed for 24 hours and left an almost colourless solution. This solution was used in further experiments with perfusion of the frog heart by Greene's cannula when it registered an effect which was specific and almost as strong as the control specimen of the original infusion.

Having thus found a method which gave a protein-free filtrate without significant loss of potency, efforts were directed towards the isolation from this of a pure product.

(g) The following methods were employed.

500 cc. of infusion of Crataegus oxyacantha were prepared as above and 25 gm. anhydrous sodium sulphate per 100 cc. added and the whole heated to 32°C, cooled and filtered. This filtrate was left overnight in the refrigerator and refiltered. The filtrate was still cloudy. 100 gm. alumina was added and the whole shaken for 15 minutes on a shaker, and filtered. The filtrate, now clear, was discarded, and the alumina eluted with phosphate-citrate buffers at the following hydrogen ion concentrations:-

a) pH 4.0

b) pH 6.0

c) pH 7.0

The elutrates were filtered, dialysed and neutralised to pH 7.0 The resulting solutions were clear and moderately free from salt. The elutrates were

tested on the perfused frog heart using the cannula of Tiegs. The only active elutrate was that eluted at pH 7.8.

20 cc. of the elutrate at pH 7.8 were evaporated to dryness, yielding .17 gm. of amorphous matter, insoluble in ether, chloroform, or alcohol, but water soluble.

As this substance was obviously impure further steps were taken as follows:-

The above quantity of infusion of Crataegus oxyacantha was prepared and 25 gm. of anhydrous sodium sulphate added per 100 cc. infusion, plus 25 cc. sodium tungstate solution and the steps repeated as above described.

Of this elutrate, 10 cc. in volume was extracted in a separator funnel with 10 cc. of petroleum ether, 10 cc. of methyl ether, 10 cc. of chloroform, and 10 cc. of acetone in turn. These were evaporated and the residues taken up in saline and tested on the frog heart with Tiegs' cannula, but were inactive.

To the reidue of the elutrate moderately strong sulphuric acid was added and allowed to stand for 2 hours. The whole was then centrifuged, and the precipitate (1), a brown amorphous gummy mass dissolved in weak alkali and the filtrate (2) kept.

The precipitate (1) in alkali was neutralised, when a grey precipitate (a) came down. This was filtered off and the filtrate (b) kept.

The filtrate (2) was shaken with 5 gm. alumina for 40 minutes and filtered (c) and the alumina eluted at pH 7.8 (d).

(a), (b), (c), and (d) were tested on the frog heart by perfusion through the cannula of Tiegs (1934). They were inactive.

(h) Considering the knowledge obtained from these facts the following method was adopted.

50 gm. powdered dry whole fruits of Haw in 400 cc. of distilled water was refluxed for 4 hours. This was centrifuged for 10 minutes, leaving 325 cc. of brown opaque fluid. 100 gm. anhydrous sodium sulphate was added to the supernatant fluid, heated to 32°C cooled and filtered. The whole was left in the ice chest overnight, centrifuged and 100 gm. alumina added and shaken for 15 minutes and centrifuged. The alumina was eluted at pH 7.8 with a phosphate- citrate buffer and centrifuged, re-eluted and re-centrifuged. The elutrates were mixed and acidified with sulphuric acid. To this was added 25 gm. alumina, shaken for 15 minutes and centrifuged. To the supernatant fluid was added another 25 gm. of alumina, shaken and re-centrifuged. The alumina was eluted at pH 8, centrifuged and re-eluted and centrifuged. The elutrates were mixed and brought to a pH 7. This mixture was tested on the frog heart: it was potent.

The remainder of the elutrate mixture (15 cc.) was

evaporated to dryness and the residue .37 gm. of amorphous brown material extracted with ether, and the ether extract evaporated to dryness. There was no residue, thus there is no ether soluble principle.

The residue, which had been unaffected by ether, was dissolved in water and dialysed, and re-evaporated to dryness. .12 gm. of a slightly coloured powder were left. This was extracted with absolute alcohol, which removed the trace of colour and left .097 gm. of colourless material. This was dissolved in Ringer's solution and tested on a perfused frog heart.

It had no effect.

CONCLUSIONS.

The final conclusions of this attempt at the isolation of the active principle of Crataegus oxyacantha is that this substance is not an alkaloid or a glycoside but is a water soluble body of unknown constitution, so far unisolated. It is insoluble in petroleum ether, methyl ether, chloroform, alcohol, benzol and acetone and is easily destroyed, presumably by oxidation, at strongly alkaline or acid pH. It is not the crataegic acid of Baechler, nor is it amygdalin or other cyanogenetic glycoside. It exists in the pulp of the fruit but not in the seeds. It is also present in the flowers but not in the wood or twigs.

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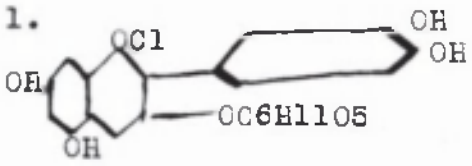
Certain tentative conclusions were drawn concerning the interesting plant pigments found in the skin of the fruits of Law. Karrer and Helfenstein (1932) have studied the pyrane and quinone pigments. They report concerning the anthocyanins as follows - " the constitution of the anthocyanidins, the basic substances of most of the red and blue berry pigments has been substantiated by synthesis, and several have been prepared which seem to be identical with natural products".

The most important anthocyanins and anthocyanidins which were prepared in 1931 are as follows:-

1.



Chrysanthenine chloride; Kurakami, Robertson, and Robinson (1931).

2. 4-B-glucosydil pelargonidin chloride.
Leon, Robertson, and Robinson.(1931).

3.Idaein chloride.
Grove and Robinson (1931).

Prunicyanin is most probably a cyanidin-3-bioside like most of the others.

Robertson and Robinson (1931) have developed a method of analysis on small quantities of the pigments. A resume of this is as follows:-

A Colour of Solution.

Anthocyanidins can be identified by comparison

of their solutions in 1% HCl. The influence of accompanying substances or co-pigments however, interferes with the colour reactions, affecting the shade and intensity of the colour. eg.:-

venin plus tannin = blue ++

cyamin plus 2 glucosidoxyxanthone = blue ++

mecocyanin plus 2 glucosidoxyxanthone = no effect.

B. The stability against oxidation provides a characteristic difference between compounds with a free hydroxyl group in position 3 and those in which this hydroxyl is substituted. The oxidation may be carried out with air in alkaline solution or with peroxide. (Karrer 1927).

C. The determination of the partition coefficient between water and organic solvents.

A characteristic for the anthocyanidins is the number of volumes of benzene which is necessary to force the anthocyanidin which is distributed between one volume of amyl alcohol and three volumes of 0.5% HCl completely into the aqueous layer. (Nolan and Casey 1931).

In Crataegus oxyacantha the fruit skin, which is bright red when fully ripe gives an acid solution when infused with distilled water, which is pink or light brown according to the concentration and the length of infusing. On rendering it alkaline a light blue colour results. This colour change with change

of pH shows particularly clearly if the infusion be shaken with kaolin and the kaolin be then rendered alkaline with sodium carbonate solution.

Proceeding on the lines of Robertson and Robinson's technique the following experiments were carried out.

1. 10 cc. of infusion plus 2 cc. tannic acid solution, the pink colour became intensified and changed to brown.

2. 5 cc. infusion plus 2 gm. sodium sulphate shaken up plus 3 cc. hydrogen peroxide - no change in colour.

3. 25 cc. infusion plus 5 gm. sodium sulphate oxidised by passing a stream of air for three hours - no change in colour.

4. Partition coefficient - this proved unsuccessful as the infusion proved too impure.

5. pH of colour changes as determined by the use of a phosphate-citrate buffer range.

(a) pH 7.5 acid:- pink
alkaline:- blue

(b) pH 5 acid:- pale yellow
alkali:- vinous red

There would seem to be at least two substances present which act as indicators, giving the colour changes listed above at these pH figures.

A substituted hydroxyl is more oxidisable than a free hydroxyl, which would therefore seem to have been present.

No further investigations were carried out on the

plant pigments present in the fruits of Crataegus oxyacantha, which would seem to be anthocyanins.

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PRESENCE OF A DIGITALIS-LIKE EFFECT FROM LIQUID EXTRACT AND TINCTURE OF CRATAEGUS OXYACANTHA.

PHARMACY.

The preparations made use of were the infusion and the tincture, prepared as follows, according to the directions of the British Pharmacopeia of 1932.

1. The infusion of Crataegus Oxyacantha.

100 gm. of powdered dry whole fruit of Haw was infused for 15 minutes with one litre of boiling glass-distilled water with three stirrings, in a porcelain pot with the lid on , and filtered hot on a Buchner filter. The fluid so obtained was measured in a volumetric cylinder and adjusted with stock solutions of the appropriate salines to have the same saline content as frog Ringer solution (Howell).

The frog Ringer solution had the following composition as made up.

Frog Ringer (Howell).

In one litre glass-distilled water dissolve:-

NaCl 7 gm Shake up: Ca added last.

NaHCO3.3 gm.

KCl .030 gm.

CaCl2 .26004 gm.

2.Tincture of Crataegus Oxyacantha.

This was prepared as follows:-

2.5 kilos of powdered dry whole fruit of Haw was placed in a large glass vessel with 5 litres of 70%

methyl alcohol and macerated for 7 days, with occasional shaking. The fluid was drained from this mass, the marc pressed and the resultant fluid added to the original bulk. The whole fluid was left for 24 hours to settle and then cleared by filtration, bottled, and stored in the refrigerator.

Tincture of Crataegus was also prepared by the percolation process and compared with that prepared by maceration, but as it proved in no way superior, the method was abandoned in favour of the maceration process.

150 gm. of soft fresh Haw fruits was cleaned and the pulp separated from the seeds by hand. The seeds were ground and infused in the usual way. The pulp was also infused, and the infusion tested on the frog heart by perfusion through Greene's cannula when it was found that the infusion from the seeds was without activity, while that from the pulp was potent. It thus appears that the potent principle lies in the pulp only.

20 gm. of dried Haw flowers gathered in April 1930 (an early season) were infused with 300 cc. of boiling distilled water and the infusion tested on the frog heart as above. This infusion was potent. The active principle thus resides in the petals of the flower also.

20 gm. of fine twigs were ground and infused with 300 cc. of boiling distilled water and the infusion

tested in a similar manner. This infusion, while slightly toxic to the frog heart did not have the characteristic action. The active principle is thus found only in the flower and the pulp of the fruit of Crataegus oxyacantha.

Other physiological solutions used in the experimental pharmacological work about to be detailed were as follows:-

(a) Tyrode-Bayliss.

In one litre of glass-distilled water dissolve-

NaCl 8 gm

KCl .2 gm

$NaHCO_3$ 1 gm.

NaH_2PO_4 .05 gm.

$CaCl_2$ .394 gm.

(b) Frog Ringer solution Sherrington.

In one litre of glass-distilled water dissolve-

NaCl 6 gm

KCl .075 gm

$NaHCO_3$ .1 gm

$CaCl_2$ .394 gm

(c) Ringer solution for Tortoises.(Sollmann).

In one litre of glass-distilled water dissolve-

NaCl 7 gm.

KCl .15 gm.

$CaCl_2$ .25 gm.

This is the perfusing fluid recommended by Sollmann (1928) for use with the Turtle heart.

PRESENCE OF A DIGITALIS-LIKE EFFECT FROM LIQUID EXTRACT AND TINCTURE OF CRATAEGUS OXYACANTHA.

PHARMACOLOGY.

(a) The Intact Frog.

The frogs used in this work were of the species Rana esculenta, obtained from the continent. The amphibian heart differs in important features from the mammalian in its behaviour to the cardiac glucosides, but the view has been developed that in some points the reaction of the human heart in disease approximates more closely to that of the frog's heart than to that of the normal mammalian organ. The more intimate analysis of the glucosidal relations to heart tissue is only possible in the frog, accordingly work on the frog forms the basis of any study of such a substance. The various members of the recognised digitalis series have a very uniform effect on the frog heart and as this typical "digitalis action" is well known it can be compared readily with the effect of some galenical preparation such as Tincture of Crataegus.

Protocol. 7/11/38.

2 p.m.

Four frogs of 25 gm. weight each injected with .5 cc. of Tincture of Crataegus into the lymph sac. The frogs leaped about for some time and appeared irritated by the injection.

2.5 p.m.

The frogs are now resting quietly.

2.10 p.m.

The frogs appear somewhat languid and are breathing very swiftly. They are stunned, the cerebrum crushed and the skin muscle and breastplate removed and the pericardium incised. The heart is dilated and dark with venous blood; the beat is slow and forceful.

2.20 p.m.

The ventricle is beating at half the rate of the auricles which are engorged with blood and contracting poorly.

2.40 p.m.

The ventricle has ceased to beat and is in a state of diastole. It is much enlarged and dark blue in colour. The auricles are beating feebly.

2.45 p.m.

The heart is at a standstill, all chambers in diastole.

A pin-point drawn across the ventricle causes a single, irregular contraction to pass over the ventricle in which the contractile movement can be seen to start from the site of stimulation, after which the ventricle returns to diastole.

The phase of ventricular intermissions is not always seen, depending somewhat on the dosage. It is not usually marked when the dosage is high, passing rapidly from a slow state of normal rhyrhm to a state of systolic arrest.

If the frog be stunned and the heart exposed and

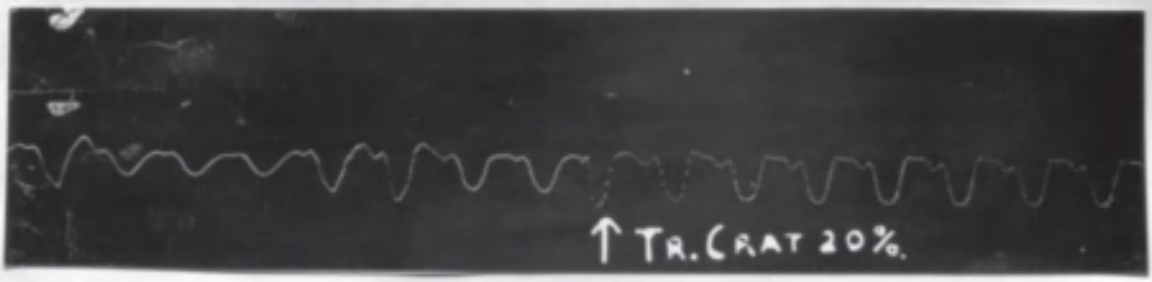


FIG. I. Action of Infusion of Crataegus in strengthening and regularising the perfused frog heart.

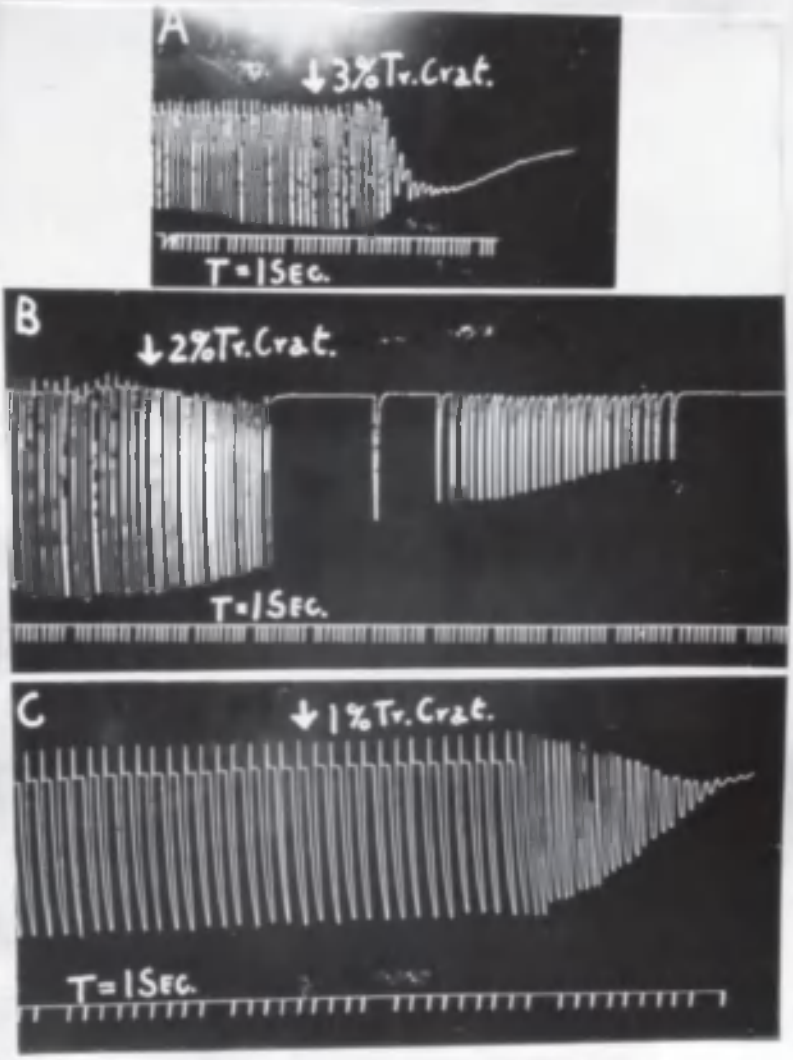


FIG. 2. Frog heart, Tieggs cannula, Frog-Ringer I8 C. A. Arrest in Systole. B. Arrest in diastole. C. Arrest in mid-position.

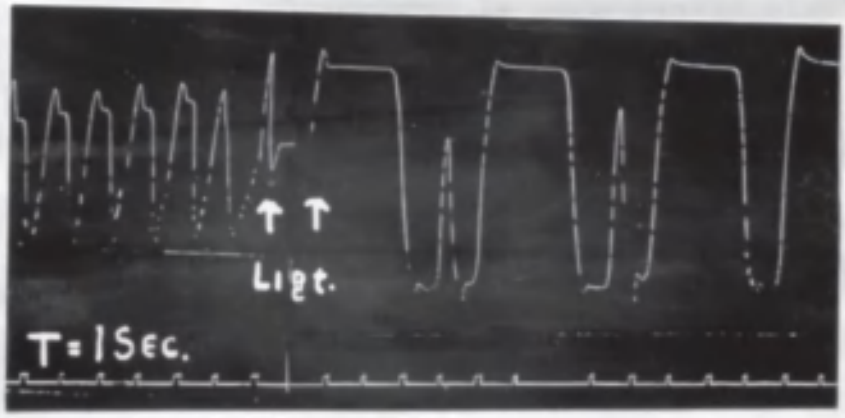


FIG.3. Effect of tying first Stannius ligt. on frog heart under the influence of Tinct. Crataegus. Increased spontaneity and coupled beats.

attached to a recording apparatus the effects detailed above can be recorded (fig. 1). Occasionally a state of " coupled beats" may be seen, as shown in fig.3 Each normal systole of the ventricle is followed by an extra-systole, while the auricle beats at twice or four times the rate of the ventricle.

If the Tincture of Crataegus be applied directly to the exposed heart arrest may occur in systole, as with large doses of drugs of the digitaloid series, but this phenomenon is more easily observed when the heart is perfused with a potent extract of the drug, while in experiments where injection is carried out by the lymph sac it is never seen, the principle of Crataegus oxyacantha being present in too small a dosage to effect systolic arrest by absorption.

If the frog heart be perfused with an infusion, or a dilution of an infusion of Crataegus oxyacantha adjusted to a saline content equivalent to that of Ringer's solution as modified by Howell (see section on Pharmacy) the effects detailed above, which are those of a small or moderate dosage of the drug are modified. The heart may be rapidly arrested in systole or in diastole, depending on the dosage administered. Fig 2 illustrates both types of arrest.

When the frog heart perfused by the well known technique of Greene with Ringer-Howell solution is perfused with a dilution of infusion of Crataegus

(which must be fresh, as it loses its potency if left exposed to the air for 24-48 hours) the heart beat may be at first slowed or unaffected in rate; sometimes it is speeded up. Later, slowing of the ventricular rate occurs. There frequently occurs a phase of marked ventricular slowing with relaxation of the ventricle, resulting in a general movement of the recording lever towards the diastolic position, and diastolic arrest of the heart may occur in this phase very early, as is seen in the illustration, fig. 1. This phase is unaccompanied by any sign of systolic contracture, and indeed occurs too early for that phenomenon to have taken place. It is removed or prevented by atropine, and is therefore merely an exaggeration of the vagal inhibition which occurs early with drugs of the digitalis series. With large doses diastolic arrest occurs in this phase, with moderate doses recovery quickly occurs and the heart beats at its previous pace or even at an increased pace. Thereafter a slow or quick process of contracture of the ventricle with ventricular intermission, slowing of the auricles and finally death in complete systole or with the ventricle in a state of partial contracture results. fig. 2. The infusion must be fresh as if it is 24 hours old it is found to lose its specific effect and be merely toxic to a variable degree.

Both the change in rhythm and the change in tone

which comprise the action in the frog heart are due to direct action upon the muscle. The nervous mechanism, extrinsic or intrinsic is not involved, for the same action is obtained after destruction of the central nervous system or division of the vago-sympathetic fibres , and in the excised heart under atropine as the following experiments show:-

Protocol. 24/10/37.

(a) Male frog 32 gm. stunned decapitated and the chord pithed; heart prepared with Greene's cannula for perfusion.

1. Perfused with Ringer-Howell solution at 16°C.

2. Perfused with Ringer-Howell solution at 16°C containing atropine sulphate 1 mgm. in 10 cc.

Rate of beating increased and size of beat augmented.

3. Perfused with infusion of Crataegus: specific effect recorded, ending in systolic arrest.

(b) Male frog 30 gm. stunned decapitated, heart excised and put in 20 cc. of Ringer-Howell solution at 16°C cotaining 2 mgm. of atropine sulphate.

Beat vigorously and regularly.

Transferred after 5 minutes to 5% infusion of Crataegus oxyacantha in Ringer's solution.

Beat for some time, slowed down and then the ventricle stopped in systole, and some time later the auricles. The ventricle was by now in systolic contracture.

(c) Male frog 36 gm. stunned decapitated and the chord pithed. Heart prepared with Greene's cannula.

Perfused with frog Ringer-Howell.

Normal beat recorded.

Curara (B.W.&Co.) 1/12 gr. in 25 cc. of Ringer's soln. 1 cc. applied to outside of heart, then with Ringer-Howell containing 5 cc. of above solution in 100 cc.

No effect was apparent although the curara solution had been previously proved potent by its action in inhibiting the contraction of a frog muscle-nerve preparation previously set up.

Perfused with infusion of Crataegus, resulting in slowing of the rhythm and augmentation of the beat and finally stoppage in diastole and the subsequent development of systolic contracture.

Both the forms of arrest, systolic and diastolic, arise from changes in the musculature, in the first the general contractile tissue being chiefly involved, while the diastolic standstill arises from action on the conducting fibres between the auricle and the ventricle and the sinus and the auricle.

Protocol. 27/11/37.

Male frog, 37 gm. perfused Greene's cannula.

Perfused with frog Ringer-Howell solution.

Rate 45 per minute; A-V interval .40 sec.

Perfused for 15 minutes with 33% infusion of Crataegus in saline.

Rate 26.5 per minute; A-V interval .70 sec.

The prolongation of conduction time is marked in this experiment.

The frog heart poisoned with Crataegus oxyacantha exhibits spontaneous beats or extra-systoles not infrequently, indicating a tendency to spontaneous contraction in a normally passive part of the heart. This phenomenon is the explanation of the experiment of Schmiedeberg (1875) where if the heart be perfused until systolic standstill occurs and the fluid pressure in the cannula then be raised by elevating the Marriott's flask, the ventricle responds by a series of contractions and may go on beating for a considerable time. The increase in spontaneity of the ventricular muscle causes it to react to the stretching of the ventricular wall by contraction.

This increase in spontaneity is also convincingly demonstrated by the fact that if the first Stanniua ligature be tied between the auricle and sinus of a frog heart under the influence of Crataegus oxyacahtha the heart does not at once cease to beat, but on the contrary assumes at once a new rhythm initiated by the auricle. (fig 3).

In addition to the change in rhythm the individual beats undergo modification under Crataegus. In the recorded heart perfused with a low concentration of infusion of Crataegus oxyacantha the systole is seen to be more complete at first without any change in the extent of the relaxation and often without any change in the rate of the pulse. The phase of increased contractility may be prolonged until it is interrupted

by the intermissions of the ventricle. The increased strength of ventricular contraction is accompanied by an increase in the speed of contraction so that the lever of the recording apparatus traces a more verticle line on the smoked paper.

The phase of increased strength of systole is soon complicated by a diminution in the relaxation of the heart so that the line of diastoles of the record falls from its base line. About this time the extent of systolic contraction becomes less also, so that the lines of systolic and diastolic extremes approximate. More often diastole remains more adequate than systole but occasionally the reverse is seen, or an equal degree of shortening of both.

The progressively deficient diastole arises from the increased slowness of relaxation, the phase of contracture having begun. The relation of this phase to that of ventricular intermissions is variable but it is usually seen that with weak concentrations of the drug intermissions occur and the heart stops in diastole, later developing contracture, while with stronger concentrations the heart develops systolic contracture before intermissions are seen. If however the heart developing contracture is slowed by heart block the extent of systole at once is increased to a marked degree as greater time is given for relaxation by the intermission.

The auricle of the frog heart reacts to Crataegus

oxyacantha in a fashion similar to the ventricle. In the beginning the strength of contractions is increased, the excursions then become smaller, apparently due to partial contracture. The auricle continues to beat in unison with the sinus until late in the poisoning, long after partial block has occurred in the auriculo-ventricular junction, and when the ventricle has come to a standstill continues to do so for some time. No contracture is seen in the auricle when the drug is perfused through the whole heart and the contrast between the white systolic ventricle and the distended purple auricles is marked.

The contracture of the ventricle often strikingly advances after stimulation of the arrested ventricle by mechanical means. Sometimes this contracture is seen to arise at the point of stimulation and to spread slowly over the surface without any real beat of the ventricle occurring.

The irregular contraction sometimes known as "the phase of peristalsis" is due to partial contracture: one part of the ventricle is unable to relax quickly enough to maintain the rhythm, but succeeds in doing so in the couse of two cycles, while another part now fails to relax, and this alternation of activity in the two halves throws the blood from side to side of the chamber without expelling it into the aorta.

Once contracture is developing or has developed

in the perfused frog heart under Crataegus the efficiency of the muscle is naturally lessened and the output must fall. In the early stages with small doses the strength and exsursion of the beat of a normal, well-nourished heart may not be much affected, but the poorly nourished heart responds with a great increase in the work done (fig. I.) while if such an irregularity as the presence of extra-systoles is noted the exhibition of the drug may remove them, regularise the rhythm and strengthen the beat.

A weak solution of the infusion of Crataegus may have no effect for some time after perfusion into the frog heart, but ultimately acts upon it in a characteristic way; but above a certain concentration of the drug the increase in strength of the drug does not shorten the time required to cause death.

Protocol. 27/11/37.

Two male frogs, weight 27 gm. prepared with Greene's cannula for heart perfusion.

1. Perfused with infusion of Crataegus.

Death in systole in 45 secs.

2. Perfused with 66% infusion of Crataegus in saline.

Death in systole in 44 secs.

If however the drug be in a lesser concentration when first applied it causes a slowing of the heart and a great diminution of systole which may not be immediately fatal but passes into a phase of increased ventricular systole and ultimately contracture.

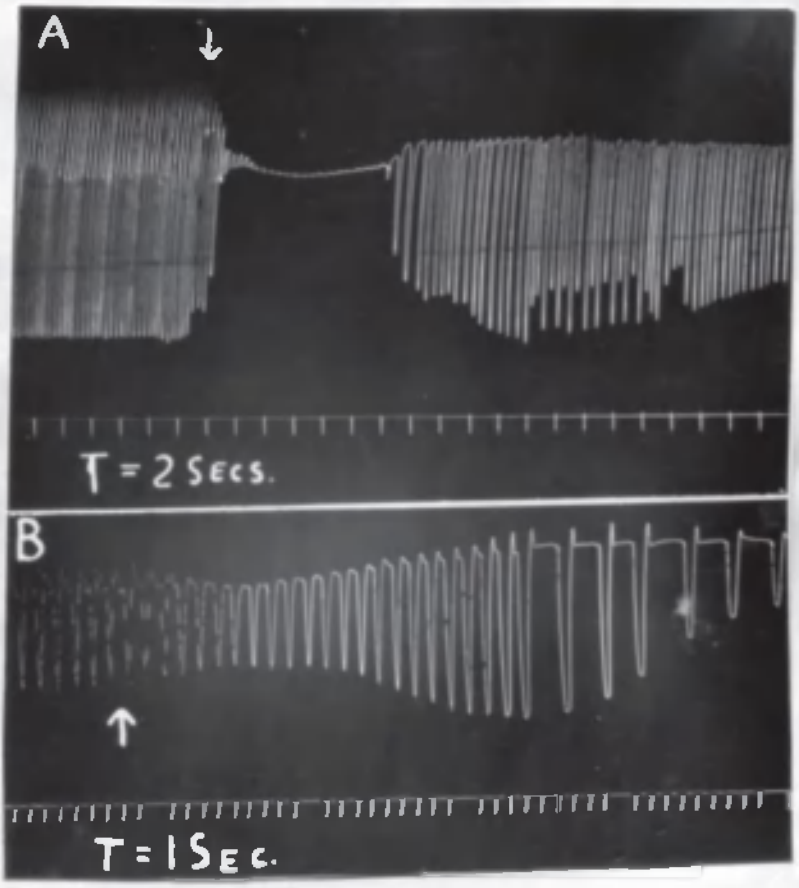


FIG. 4. Diastolic arrest of frog heart with spontaneous recovery. Greene's cannula. Frog Ringer 20°C. A. Complete arrest. B. Partial arrest. 33% Tincture of Crataegus added at arrow.

Protocol. 3/12/37.

Two male frogs, 32 gm. Prepared with Greene's cannula for perfusion.

1. Perfused with infusion of Crataegus.

Death in diastole in 15 secs.

2. Perfused with 33% infusion of Crataegus.

Exhibited phase of diminished systole and slowing (vagal action) followed by recovery and slow vigorous beats.

Perfused at this stage with infusion of Crataegus. Slow vigorous systoles maintained for two minutes followed by progressive contracture and death in systole in 3 mins. 7 secs. (fig 6).

It is thus obvious that Crataegus oxyacantha has a preliminary powerful action on the parasympathetic inhibitory mechanism and a later, less quickly developed action on the muscle fibres of the heart which leads to arrest of the ventricle in systolic contracture. It is in the vigour and degree of this preliminary action that Crataegus differs from Digitalis in its action on the perfused frog heart, and the the degree of recovery from it which is possible (even from complete standstill of the heart). See fig. 4

This action is not reversible but continues in the perfused heart when an attempt is made to wash out the drug with saline. The preliminary stage of vagal inhibition which with a small dose of the drug leads to spontaneous recovery, but with a larger dose is the cause of early arrest, often in

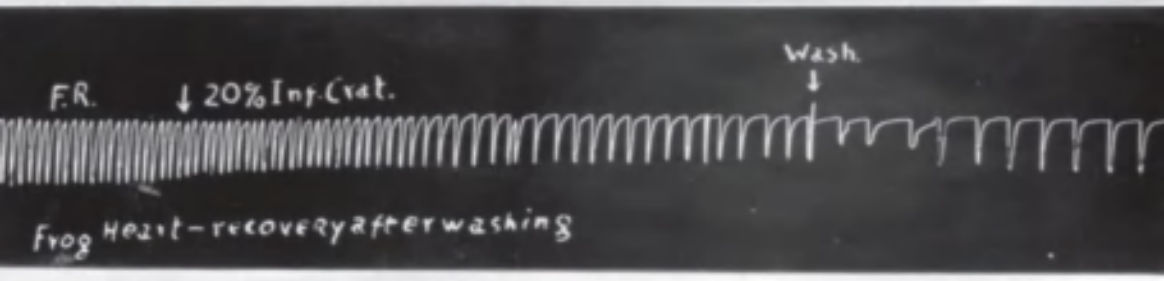


FIG. 5. Non-Reversibility of effect of 20% Tinct. Crataegus by washing with saline. 25 gm. male frog. Greene's cannula. 19 C.

diastole, and which is not seen in the heart under atropine, may be rapidly reversed or removed by washing with saline; a larger dose of Crataegus which has begun to affect the muscle fibre may not be so affected. (Figs).

The irreversibility of the action of Crataegus oxyacantha on the frog heart is demonstrated by the classical experiment of Issikutz (1915).

Protocol. 12/1/38.

Four male frogs of 30-31 gm. weight. Prepared with Greene's cannula for perfusion with Ringer-Howell solution at 14 C.

(a) Perfused with 1/50 Diginutin (B.W. & Co) solution in Ringer.

Death in 5 mins. 40 secs.

(b) Perfused with 1/50 Diginutin (B.W.& Co) solution in Ringer for 3 mins., Ringer-Howell for 10 mins., and then the same solution of 1/50 Diginutin till death.

Death in 7 mins.

(c) Perfused with infusion of Crataegus.

Death in 6 mins.

(d) Perfused with infusion of Crataegus for 3 mins., Ringer-Howell 10 mins, and Crataegus till death.

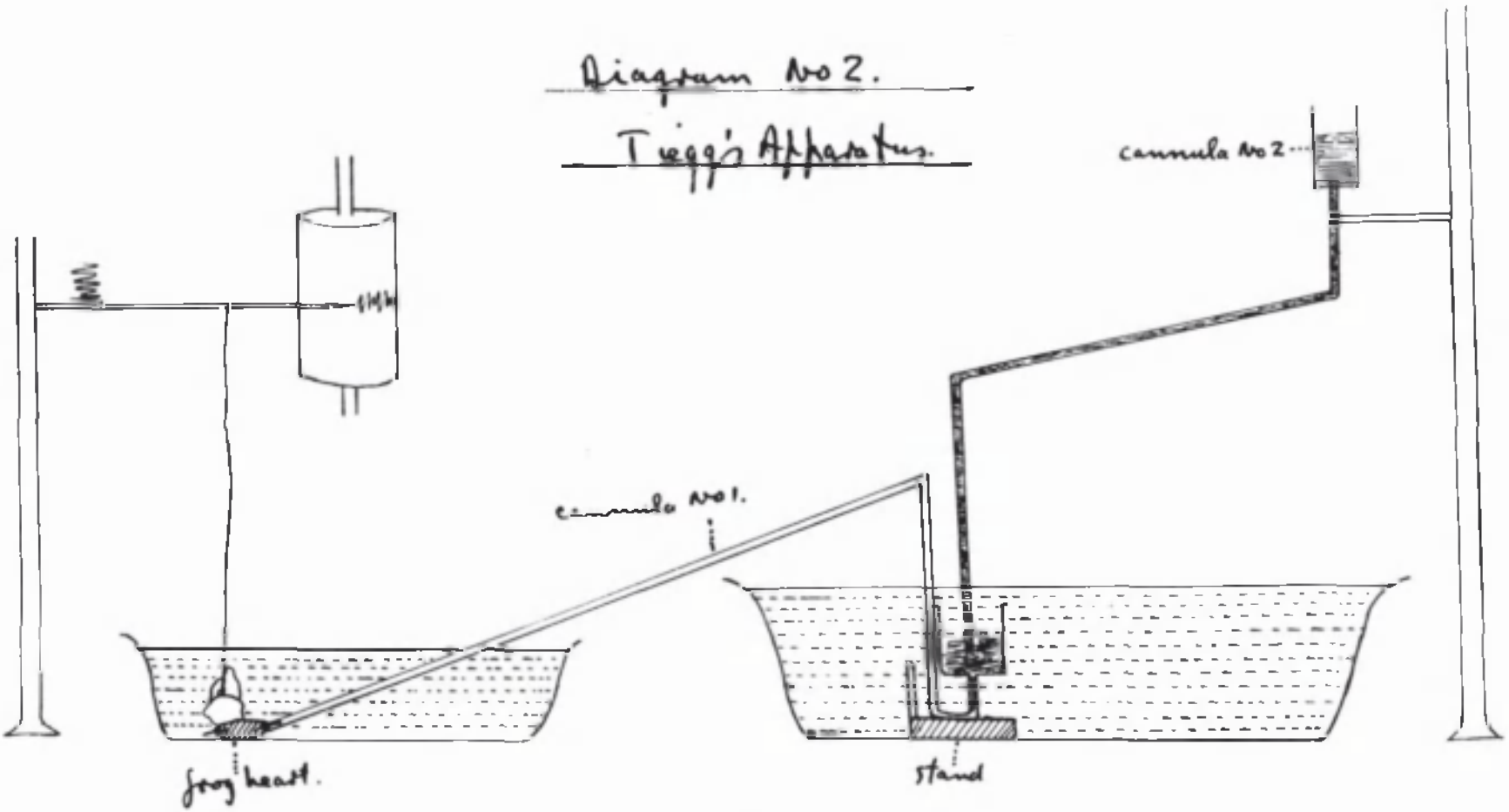
Death in 5 mins.40 secs.

There is thus evidence of more accumulation of Crataegus in the frog heart than of Digiutin, a stable preparation of the whole glucosides of Digitalis.

That the action of Crataegus oxyacantha on the

Diagram No 2.

Trigg's Apparatus.



frog heart is entirely the same as that of the "digitaloids" is shown by the results of an application of "the combined ouabain technique" of Hatcher and Brody (1923) to the frog heart perfused through Riegs' cannula (1934).

Protocol. 7/11/38.

Four male frogs of the same weight to within a gram were chosen and prepared as follows:-

Each frog was stunned, decapitated and pithed, skinned, and the lower part of the body with the bowel and in a female the ovaries removed, and the fore-legs, breast plate and pericardium removed also. This left the back part of the thoracic cage with the heart, liver, stomach and lungs, which were pinned down to a piece of cork attached to the bottom of a circular 150 cc. porcelain dish by sealing wax or plasticine. The dish was filled with frog Ringer-Howell solution and the lungs removed if they floated up and tended to interfere with the heart. A clot of blood which frequently forms at the pyloric end of the stomach may also be removed. It is advisable also to rinse the preparation in saline before filling up the dish as the saline in it may become blood stained. A thread was passed round the vein leading from the liver to the venous sinuses and the cannula inserted. The cannula must be completely filled with saline in order that air may not enter the heart, and the reservoir kept under the saline filling the larger dish.(1 litre size). See illustration. Diagm 2

The cannula was tied into place, the aortae snipped and the proximal end of the cannula set in a little wooden stand. The difference of level between the two dishes kept a constant stream flowing through the cannula and if both dishes be filled completely this is practically constant as the loss via the cannula is negligble compared to the total volume of the larger dish. As the heart was kept in saline it did not dry. The tip of the ventricle may be attached to a recording lever in the usual way. The perfusion of solutions of drugs was accomplished by filling the feeding cannula and attaching it to its stand and applying it to the reservoir of the perfusion cannula, when the drug flowed through undiluted. If colouless drug solutions are used the rate of flow must first be calibrated by the use of some non-toxic dye stuff.

The frogs, perfused with Ringer-Howell at 16°C were treated as follows:-

1.Male frog, 31 gm.

Perfused with 1/100,000 g-strophanthin (ouabain) soln.

Death in 5 mins. 10 secs.

2. Male frog, 31.5 gm.

Given .0140 gm. ouabain in 1 cc. saline 1 hr. before by lymph sac.

Perfused with ouabain in saline 1/100,000.

Death in 1 min. 40 secs.

3. Male frog, 31.3 gm.

Perfused with infusion of Crataegus oxyacantha.

Death in 4 mins.

4. Male frog, 31.7 gm.

Given .0140 gm. ouabain in 1 cc. saline 1 hr. before by lymph sac.

Perfused with infusion of Crataegus.

Death in 2 mins.

This experiment proves that Crataegus oxyacantha contains a specific principle akin to that of Digitalis and that the fresh infusion is of a strength of the order of 1/100,000 g-strophanthin solution.

The temperature relations of Crataegus are somewhat anomalous. Infusion of Crataegus at 8°C applied to the haert cooled previously with saline at 8°C has very little effect, while at 30°C the rapidly beating irregular heart is affected, but only to a lesser degree than at the usual temperatures of 15-20°C.

The action of Ca is essentially different from that of Crataegus as shown by the following experiment:-

Protocol. 17/11/38.

Female frog, weight 30 gm., perfused with Ringer-Howell at 16°C and then Ringer containing excess of Ca delivered to the heart (Ca four times normal). The heart immediately went into a state of increased systolic tone with irregularity of the amount of diastolic relaxation. Slow recovery of the level of diastolic recovery and relaxation occurred followed by slowing of the heart rate from 60 per minute to 48 per minute which slowly tended to pass into contracture, but not markedly so, until death occurred in

systole. Sinus rhythm was maintained throughout.

2. Male frog, weight 39 gm., Perfused with Ringer-Howell at 16° C and then with infusion of Crataegus 50% in saline.

The heart went into a condition of increased systolic tone at once with decreased diastolic relaxation, contracture developed, then intermissions and peristalsis of the ventricle. In this phase the auricle would contract and part of the base of the ventricle respond while the apex remained in contracture, only relaxing at odd intervals. Finally the whole ventricle remained in systole with the auricles beating. After some time they stopped in diastole.

The excessive Ca ion tended to slow the heart and promote contracture but had not the specific effects in altering the rhythm.

The ELECTROCARDIOGRAM.

The effects of Crataegus oxyacantha as studied by observation and recording of the intact, perfused, and isolated frog heart were now to be correlated with the electrocardiographic picture.

A valve electrocardiograph was used with a Matthew's oscillograph and the pulse rate recorded mechanically on the pulse recorder developed by Bell et al (1930). From the input terminals of the apparatus two wires were lead, ending in soldered needles which were thrust beneath the skin of the frog on either side of the

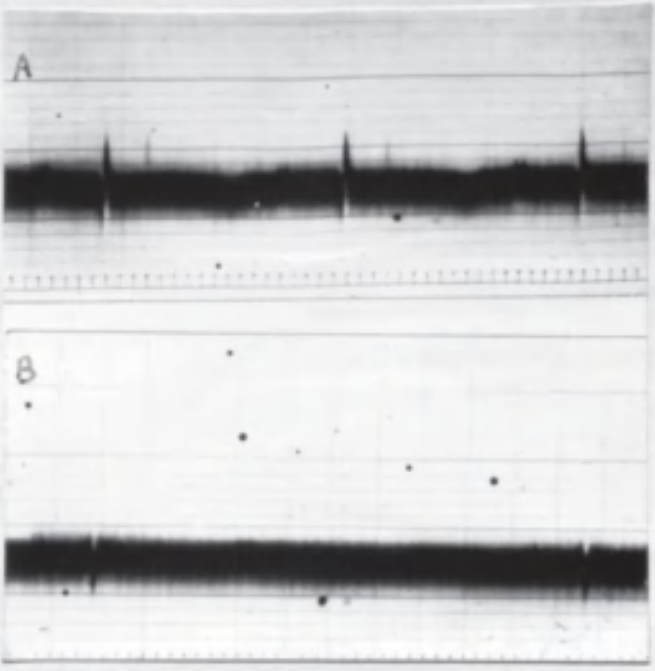


Fig. 7. Frog Electrocardiogram.

A. Before Crataegus.

B. After Crataegus.

thorax to act as input electrodes. The frog was decerebrated by crushing and pinned on its back.

The rate of the normal frog heart was 60 per min. The characteristics were as follows; P wave - ve., bifid in form, occupies .05 sec; QRS complex - ve.; PR = .228 sec.; QS = .05 sec.; R = .025 sec.; T wave is - ve.; RT = .375 sec.; T = .075 sec.
The form of the wave is shown in fig. 4.

.5 cc. tincture of Crataegus oxyacantha injected into the lymph sac of a 30 gm. male frog, after 30 mins. produces the effects shown in the figure.

The rate of contraction of the heart was now about 13 per minute.
P wave was - ve., bifid and occupies .065 sec.; QRS is - ve. and no longer bifid; PR = .20 sec.; QS=.05 sec;- R = .025 sec.; T wave is absent or only slightly distinguishable in which case it is positive and RT=.550sec

The rate of beating is thus greatly slowed, the voltage and sign of the QRS complex inverted and reduced and the T wave inverted or rendered indistinguishable. The PR interval is shortened.

The effects of Crataegus on the frog heart are thus centred chiefly on the muscle rather than on the conducting system.

THE TORTOISE.

The Tortoise (Chrysemys elegans) provides a convenient cold-blooded animal on which to compare and contrast the action of Digitalis and its allies with the action on the frog. The matter has been investigated by Botazzi (1901), Fanno (1888) and Gruber (1921).

According to Gruber there occur in the auricle of the tortoise rhythmical variations of tone which are lessened by Digitalis as a result of induced contracture. The phase of diastolic pauses may occur as in the frog and the effect is readily reversible by washing out with saline.

Protocol. 7/6/38.

A tortoise was killed by stunning and decapitation and the shell opened by saw cuts and removed on the ventral aspect. The heart was excised and circular sections cut from the auricle and ventricle, and prepared according to the technique of Sollmann.(1928).

In this preparation the circle of cardiac tissue is opened out to form a strip and one end attached to the clip of a recording lever and the other to the glass aerating cannula of a mammalian bowel bath. The suspension is aerated and the solution used is as follows:-

NaCl 7 gm.

KCl .15 gm.

CaCl2 .25 gm. per litre of distilled water.

The nutritive fluid was at room temperature (19C). A slowly moving drum was used. The strips from the auricle and ventricle were arranged so as to record one above the other, and suspended in the one bath. Tortoise A.

A record of the normal cardiac contraction of the strips showed no rhythmical variation as described by Gruber. The rate was 12 beats per minute for auricle and ventricle.

To the bath of 200 cc. was added 10 cc. of infusion of Crataegus to give a concentration of 1 in 20. The ventricle was slowed in rate and the size of excursion of the lever is. the degree of systole increased, ventricular extra-systole appeared and the ventricular strip stopped in diastole while the auricular strip was slowed to 11 beats per minute. Later the auricular strip was slowed further to 6 beats per minute. The force and degree of systole were progressively lessened. Extra-systole occurred.

At this stage, with the ventricle in diastolic arrest and the auricle beating 6 times per minute the fluid was changed and washed twice with saline. Recovery occurred and was maintained with an auricular rate of 4 beats per minute and a ventricular rate of 10 beats per minute.

Tortoise B.

A similar preparation was made.

A normal tracing was made and one cat unit of Digitalis administered.

Similar results occurred: recovery by washing with saline was even more complete.

After recovery 2 mgm. ouabain in 200 cc. saline were administered.

Similar results occurred again.

The tortoise heart reacts to Crataegus oxyacantha in a manner similar to its reaction to Digitalis and Strophanthin. The reaction to Crataegus is reversible by washing with saline, probably due to the fact that the slow metabolic processes in the heart of this animal permit of reversal by washing before the principle has become fixed in the tissue and is as yet exerting its influence by means of cell surface phenomena. The ventricular strip of such a preparation is more sensitive than the auricular strip, and the action may be more thoroughly reversed.

THE ELECTROCARDIOGRAM.

As in the case of the frog these actions were checked on the electrocardiogram and the pulse recorder.

Wires were led off from the input terminals of the apparatus in the customary manner and the ends wrapped in cotton wool soaked in saline. These plugs were inserted into the animal at the base of the neck anteriorly and beside the rectum, while the animal, minus the ventral part of its carapace, lay

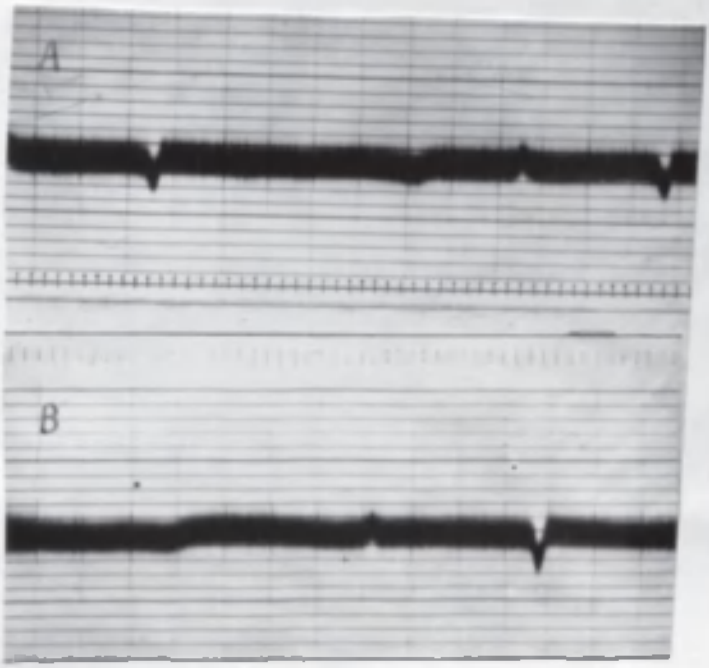


FIG. 9. Tortoise Electrocardiogram.

A. Before Crataegus.

B. After Crataegus.

on its back. This arrangement gave a longitudinal lead.

The normal electrocardiogram of the of the tortoise is as shown in fig. 9.

The rate per minute = 25 beats.

PR interval = .6 sec.

QS " = .05 sec.

RT " = 1.1 sec.

T occupies 0.1 sec.

P " 0.05 sec.

P wave is vertical and fairly sharp; QRS is inverted, of low voltage, and as a complex rounded; T is inverted and indistinct.

Tincture of Crataegus .5 cc. injected into the great vein entering the sinus affects the electrocardiogram as follows:- See fig. 9.

Rate per minute = 18.3 beats.

PR interval = .73 sec

QS " = .08 sec

RT " = 1.2 sec.

T occupies .2 sec

P " .07 sec

The P wave is vertical but not so sharp as in the normal; the QRS complex is inverted, of increased voltage, and sharper in outline; The T wave is inverted and less pronounced.

4 mgm. of ouabain in 2 cc. of saline intravenously had a similar effect on the electrocardiogram of

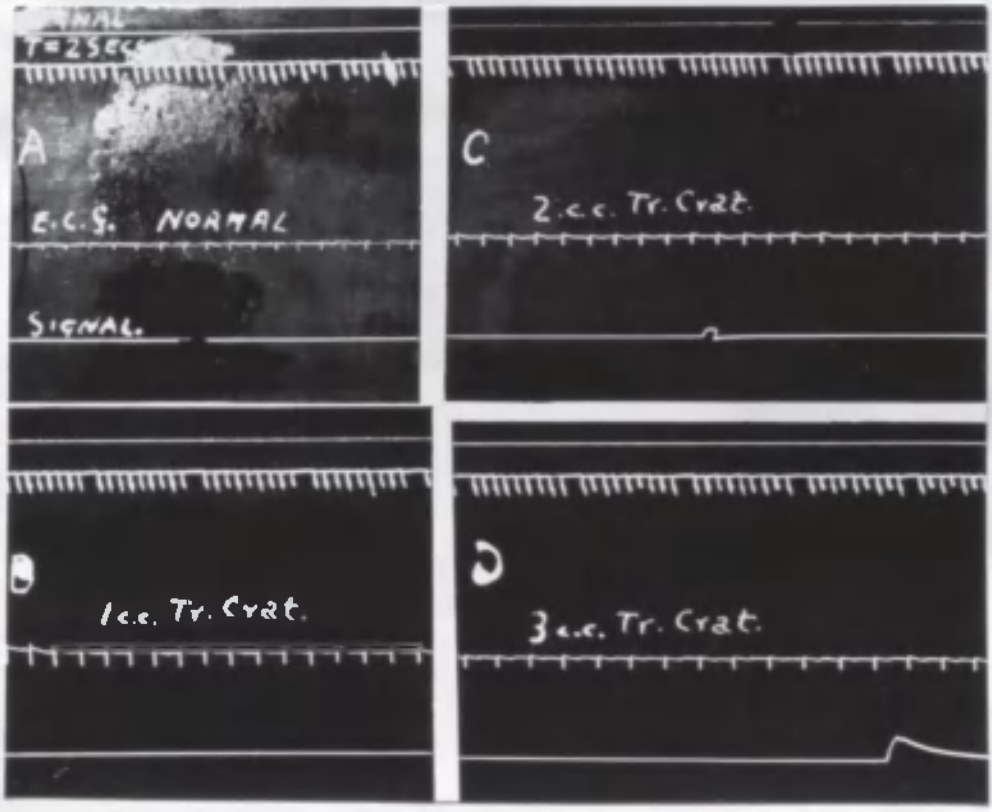


FIG. IO. Tortoise Pulse Record.

A. Normal.

B. After I cc. Crataegus - augmentation.

C. After 2 cc. Crataegus - slowing.

D. After 3 cc. Crataegus - further slowing.

another tortoise, but the slowing was not so pronounced and the complex was disturbed in that a positive S wave was flattened out.

The corroborating recording of the effects on on the rate and voltage of the pulse recorder of Bell et al. (1930) is appended. See fig. 10. The great increase in voltage of the cardiac contractions and the slowing of the pulse under Crataegus can be seen clearly.

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THE AVIAN HEART.

In conducting this part of the investigation the method used was that introduced by Paton (1912) and considerably employed previously (Graham 1938). This consists of recording a direct myocardiographic tracing from the heart while the bird continues to breathe with the aid of its intact cervical and abdominal air sacs.

After an intravenous injection of Tincture of Crataegus diluted with saline the first phase is introduced by a slight slowing of the beat along with an increase of the movement of the ventricle towards systole and with a more complete relaxation in diastole. The rhythm becomes slower at the same time. The change progresses gradually and is due to a prolongation of the pause in diastole. The auricle is similarly affected. These effects are removed to a great extent by atropine. Vago-section can not be performed in this preparation as respiration ceases. Atropine removes the slowing effect and results in a rate of contraction much greater than previous to the exhibition of Crataegus and with a force of beating which is less than just prior to its administration but greater than prior to the administration of Crataegus. These points are illustrated in the following experiment:-

Protocol. 27/10/38.

Drake 2.4 kilo. .75 gr. Nembutal at 2.30 pm.

.25 gr. " at 3 pm.

Some ether given.

Apparatus assembled and preparation made.

3.25 Corresponding points recorded and tracings of normal contractions taken. Rate 280 per minute.

1. Tincture of Crataegus 3 cc. in 3 cc. saline intravenously slowly.

Immediate inhibition of heart which becomes engorged and is almost arrested in diastole. Recovery is rapid and results in a few minutes in a greatly increased systolic contraction of the ventricle with increased diastolic relaxation and a rate of 240 per minute. The auricle is similarly slowed to 240 beats per minute and assumes a state of decreased tone. The rhythm is normal.

2. A further 2.5 cc. of tincture of Crataegus in saline resulted in a diminution in the extent and force of ventricular systole, the ventricle having definitely assumed a position of increased tone or systolic contracture having developed. The rhythm of the whole heart is for the most part sinus rhythm, the auricle beating in unison with the ventricle at a rate of 220 per minute, but showing some irregularity of the force of systole and at one point the record shows a missed beat and at another a ventricular extra-systole.

3. 1 mgm. of atropine sulphate intravenously removes these irregularities and results in a normal rhythm at a rate of 290 per minute which is a faster rate than the heart originally showed.

The record of the ventricular systole which

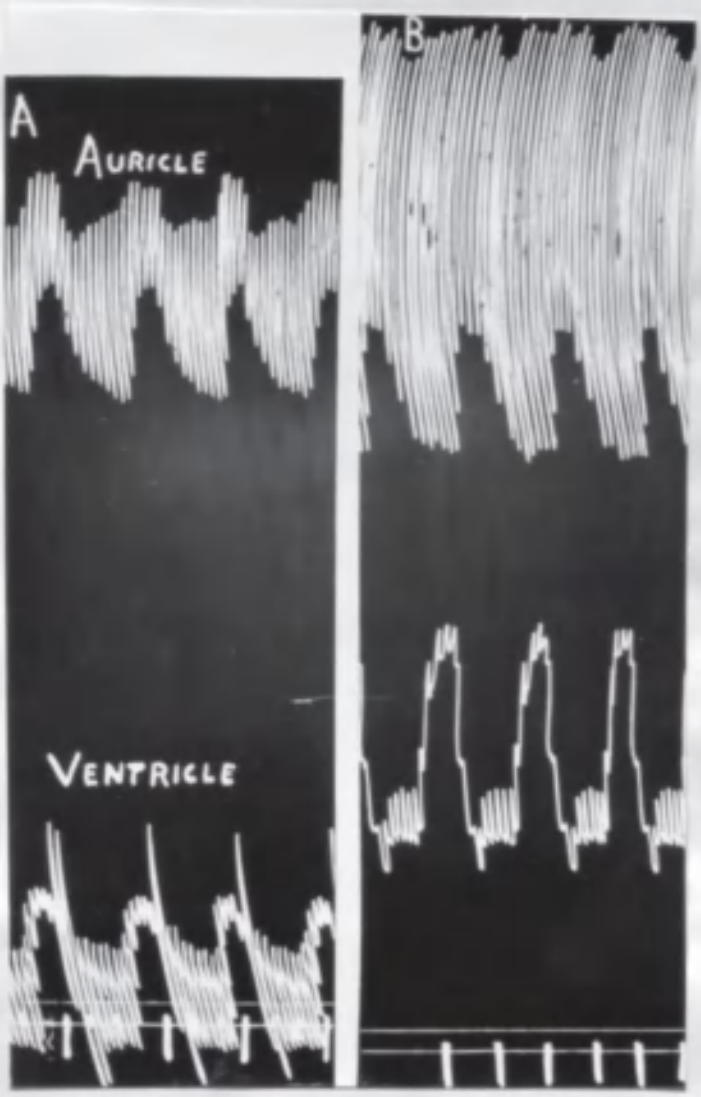

FIG. II. Drake. 2 kilos weight, nembutal I gr. Direct *Myocardiogram*.

A. Normal record.

B. 5 mins. after I.8 cc. of Tincture of Crataegus.

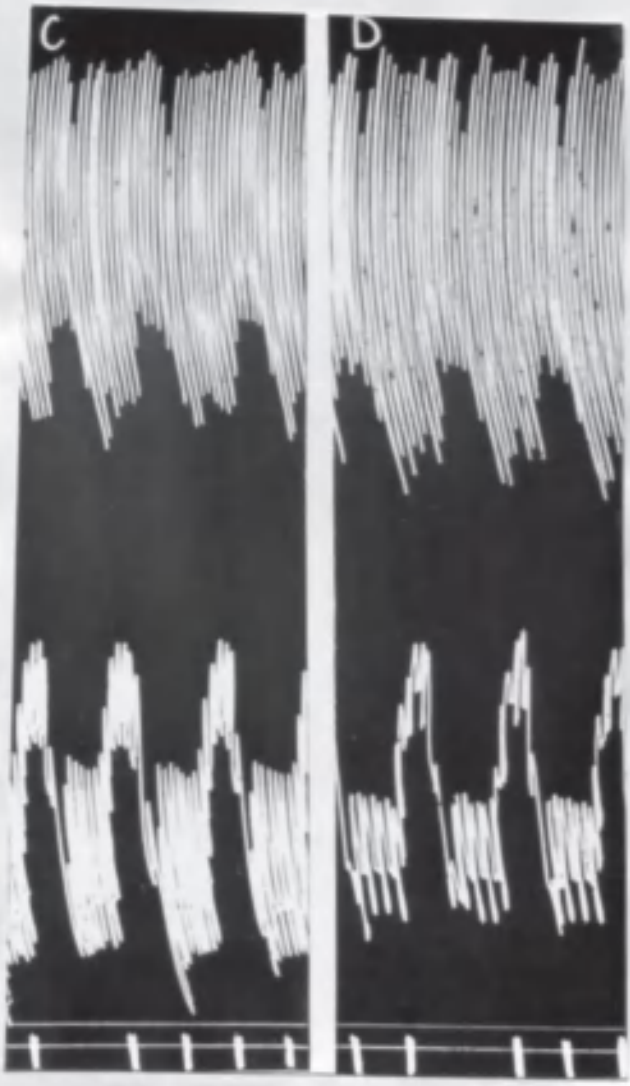

FIG. II. Drake, 2 kilos weight.

Direct Myocardiogram (contd.).

C. After I mgm. atropine sulphate/ kilo.

D. After further dosage with Crataegus.

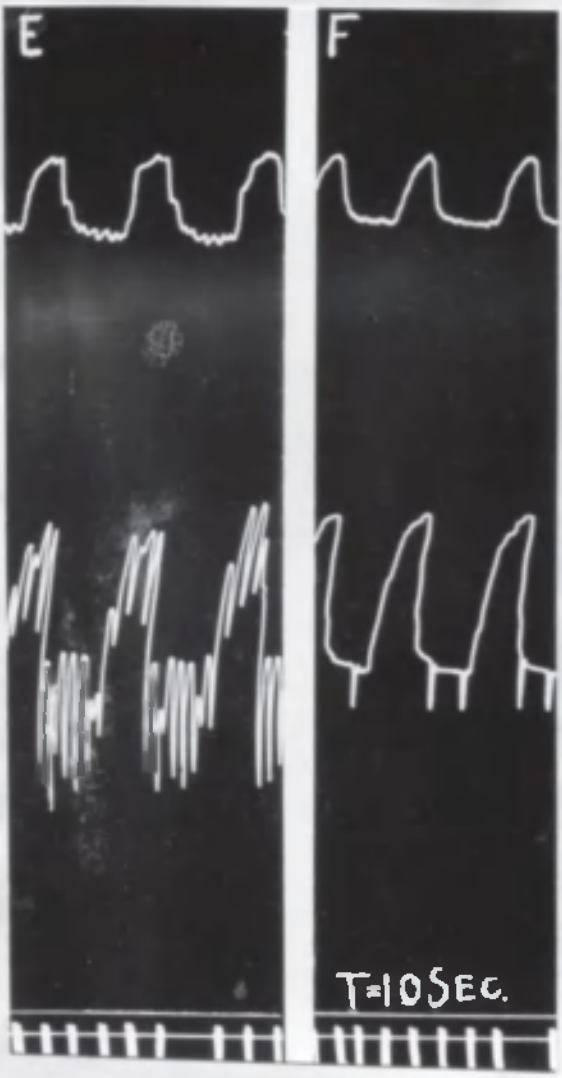

FIG. II. (contd.).

E. Inhibition of auricle.

F. Heart block.

originally gave a swing measuring 2.1 inches, developed under 3 cc. of Crataegus to 5.3 inches, fell under further dosage to 3.8 inches and under atropine measured 3.4 inches.

4. Further administration of 3 cc. of tincture of Crataegus in saline results in marked diminution in the rate of contraction to 210 per minute with irregularity of auricle and ventricle in the nature of ectopic beats of spontaneous origin but no heart block. Recovery from this effect is marked but further dosage results in continued systolic contracture of auricle and ventricle.

Unfortunately the cardiac contractions become so feeble that they will not record, but inspection of the heart which is now considerably slowed, shows the development of marked irregularities. Ectopic beats of sinus origin, fully compensated, may be seen, and marked auricular fibrillation is not difficult to watch. Heart block, rapidly developing to full block, comes on if further quantities of the drug are given. At times the whole heart intermits. Finally systolic arrest of the ventricle occurs after a phase of fibrillation, and soon the auricle ceases after a period of violent fibrillation when it sways from side to side as a whole but does not expel blood.

Some of these irregularities may be seen in fig. II.

ELECTROCARDIOGRAM.

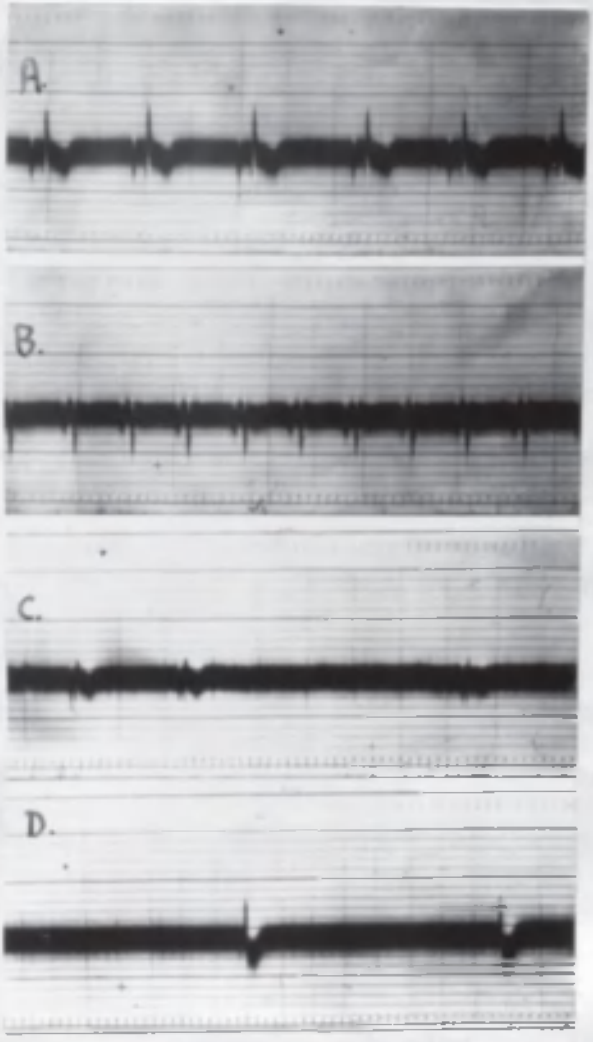

FIG. I2. Drake. 3.2 kilos weight. Nembutal.

A. Normal.

B. After I cc. Tincture of Crataegus.

C. IO mins. later.

D. Just before death.

ELECTROCARDIOGRAM.

These records and observations were supplemented by electrocardiographic records.

The bird was anaesthetised with nembutal and ether, the jugular vein exposed for intravenous administration of the drug, and the input terminals of the electrocardiograph attached to two wires soldered to needles which were thrust into the muscle of the breast on either side of the carina or keel of the breastbone, to act as electrodes.

Fig. 12 shows the normal electrocardiogram of a drake. Its main features are as follows:-

Rate 160 beats per minute.

P wave - ve. and of high voltage, coming sharply off the line: P = .02 sec.

Q -ve., R + ve., S - ve. QRS complex coming sharply off the isoelectric line and of voltage less than twice that of P. QS = .02 sec. R =?. PR = .06 sec.

T wave - ve., of high voltage and broad base. T = .08 sec.

QT = .08 sec.

The unusual features of this electrocardiogram are constantly reproduced by these birds, although the rate of the pulse may vary somewhat.

Protocol. 7/12/38.

Drake 3.6 kilo, Nembutal gr.1 intramuscularly 2 pm. Left in darkness.

3 pm. Light ether anaesthesia.

Right jugular vein exposed: bird taken to the

oscillograph room, stretched on an insulated mat on a table and input electrodes thrust into either pectoral muscle.

3.15 pm. Normal electrocardiogram recorded.

3.20 pm. Tincture of Crataegus 1 cc. in 2 cc. given intravenously. Records taken at various intervals until death.

3.21 pm. Pulse rate 140 per minute.

3.25 pm. Pulse rate 240 per minute.

P wave - ve. P = .035 sec.

QRS - ve., no longer bifid. QS = .035 sec. PR = .055 sec

T wave - ve. of much less voltage, and sharper in form. QT = .04 sec. T wave may be missing.

The P wave may be bifid and is always much less sharp, occupies a longer time and is broader at the base; the R wave is inverted, slower, of lessened absolute voltage though maintaining the same relation to P: and T of less voltage, sharper and coming much sooner after R.

3.31 pm. Irregularity of the beats is now apparent. The rate is approximately 80 per minute.

P wave is - ve. and of fair voltage. P = .03 sec.

QRS is + ve. bifid, having again changed its sign, of small voltage, less than P. QS = .02 sec. PR = .02 sec.

T wave is - ve., sharply defined and of greater voltage than before. T = .05 sec. QT = .06 sec.

3.40 pm. Most irregular beats: rate approximately 40 per minute.

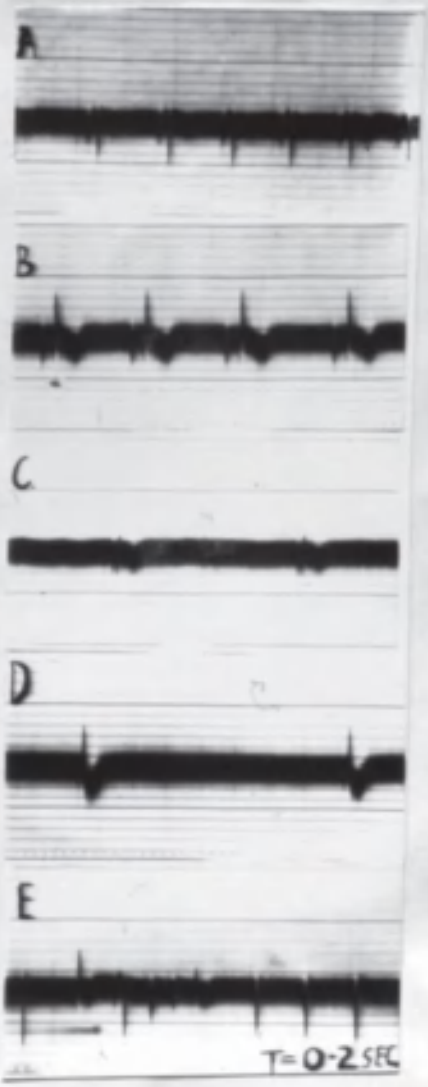

FIG. I2. (contd.).

Electrocardiogram.

A. Normal.

B. Effect of I unit of Digitalis.

C. Effect of 2 units .

D. Effect of 3 units.

E. Extra - systoles and fibrillation.

P wave - ve. Sharply defined and of low voltage.

P = .025 sec.

QRS complex + ve and of voltage the same as normally or three times its previous voltage. PR = .025 sec.

QS = .02 sec.

T wave - ve. of increased voltage and coming off the S wave in a manner suggestive of infarct. T = .075 sec.

QT = .05 sec.

P is speeded up and PR and T lengthened, while QT is also less.

3.42 pm. Death.

Digitalis has a very different effect; the PR interval is greatly lengthened, T but little affected R little affected until auricular and ventricular extra-systoles ensue with large doses and make the pulse irregular. Sinus arrhythmia becomes marked, the effect of the drug being on the conductivity of the heart and its spontaneity also.

See fig. 12.

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THE MAMMALIAN HEART.

1. IN VIVO.

The changes in the mammalian heart under the members of the Digitalis series come on so slowly, even when the drug is injected intravenously, that they can be followed only imperfectly and with difficulty with the eye. Accordingly methods of graphic recording have to be resorted to.

Direct myocardiographic recordings of the contractions of the intact auricle and ventricle were obtained by a slight modification of the method applied to the duck, artificial respiration being maintained throughout the experiment.

Protocol. 24/11/30.

Cat, male, wight 4.1 kilo.

2.5 pm. 1.5 gr. nembutal in 9 cc. saline subcutaneously.

2.45 pm. Light ether anaesthesia.

Tracheotomy performed, right jugular vein cleared and mosquito forceps applied. Skin removed from chest, breast bone and portion of the ribs raised and the stump firmly tied to prevent haemorrhage from the mammary arteries, and cut close.

Respiratory pump applied and adjusted. Pericardium incised and clipped to the edges of the wound.

Frog heart clip applied to the auricle or a ligature tied round a portion of the auricle, and a hook to the ventricle, and the tensions adjusted until

a suitable record was obtained.

3.5 pm. 20 cc. tincture of Crataegus in 20 cc. saline prepared.

3.8 pm. 2 cc. of this injected into the jugular vein slowly. After 5 minutes a record is taken and the process repeated.

The effect of this procedure was to induce an increase in the force and degree of systole and some increase in the degree of diastole, while the rate of contraction, originally 240 beats per minute, was decreased to 216 per minute.

The average length of the excursion of the lever attached to the auricle increased from 75 mm. to 100 mm. of which increase the major portion was towards systole, while the ventricular increase was from 10 mm. to 20 mm. later increasing to 30mm. (an increase of 200%), while the auricle remained as before. This increase in the degree and force of systole with relatively slight slowing (circa 15%) is remarkable.

3.45 pm. A total volume of 10 cc. of the preparation having been given, the auricle is beginning to develop a slight degree of contracture, diminishing both its systolic and diastolic excursion while the ventricle is vigorous in the extreme.

After 5 minutes a record was taken.

The rate of contraction is restored to 255 per minute while the ventricular excursion increased to 36 mm., and the auricular excursion was 60 mm.

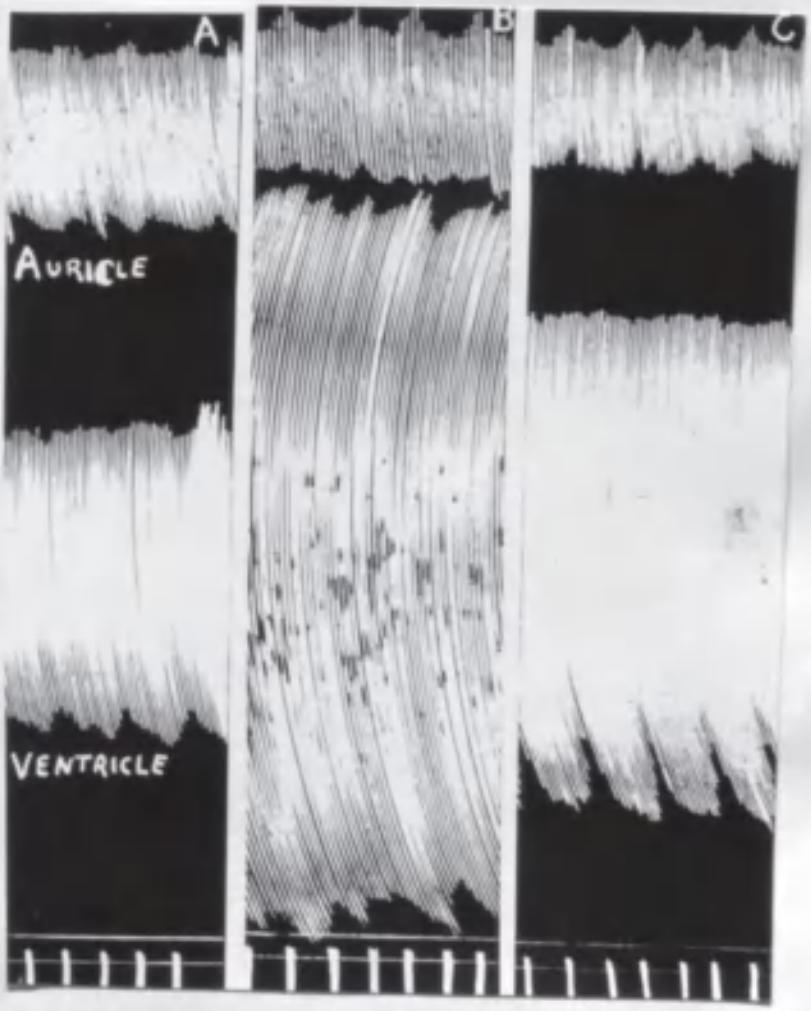

FIG. I4. Cat, male, weight 2.8 kilos.

Direct Myocardiogram.

A. Normal.

B. After I.5 cc. Tincture of Crataegus.

C. After I mgm. atropine sulphate.

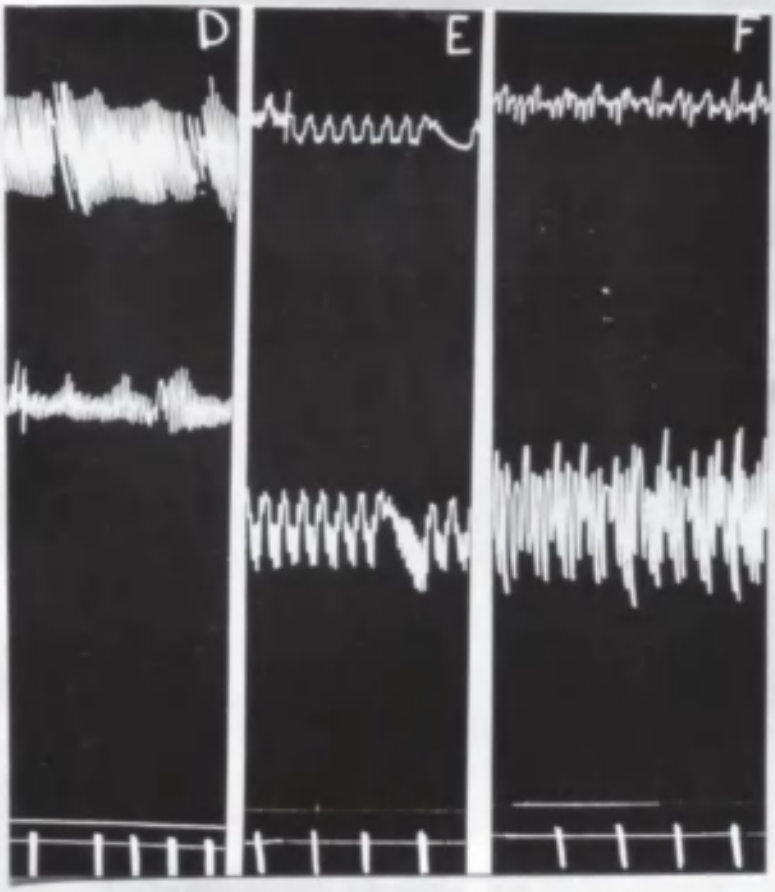

FIG. I4 (contd.).

D. Sinus irregularities of heart.

E. Ventricular tachycardia.

F. Auricular fibrillation.

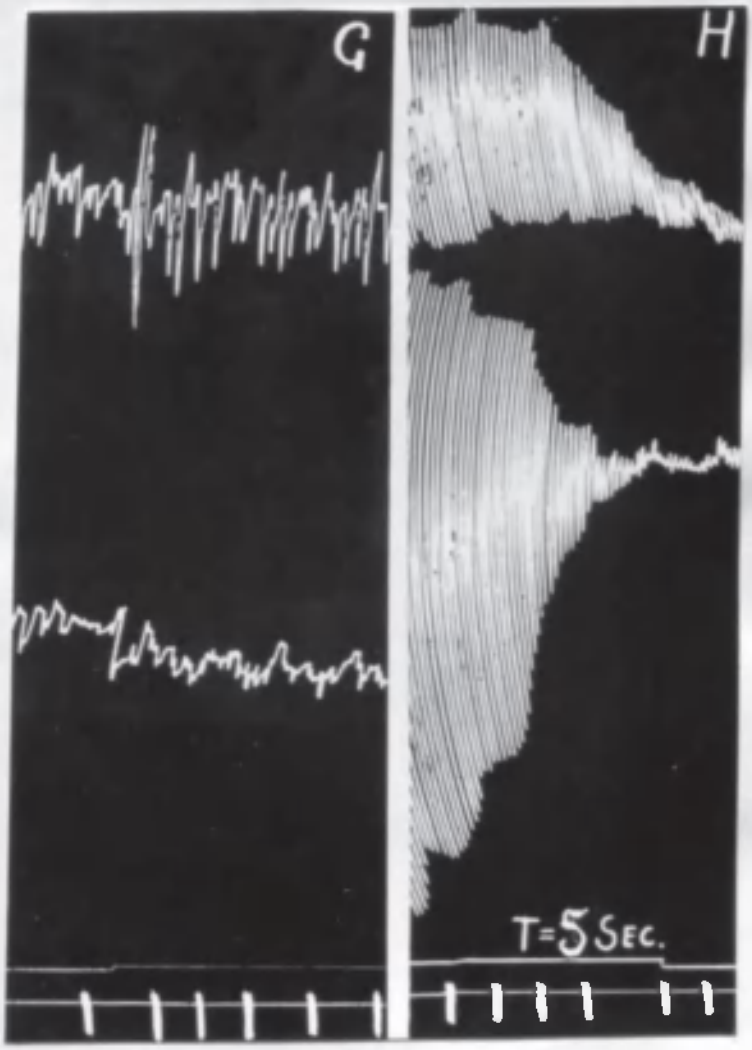

FIG. I4. (contd.).

G. Auricular fibrillation, extra-systoles and partial heart block.

H. Effect of intravenous Crataegus in vagotomised atropinised mammal.

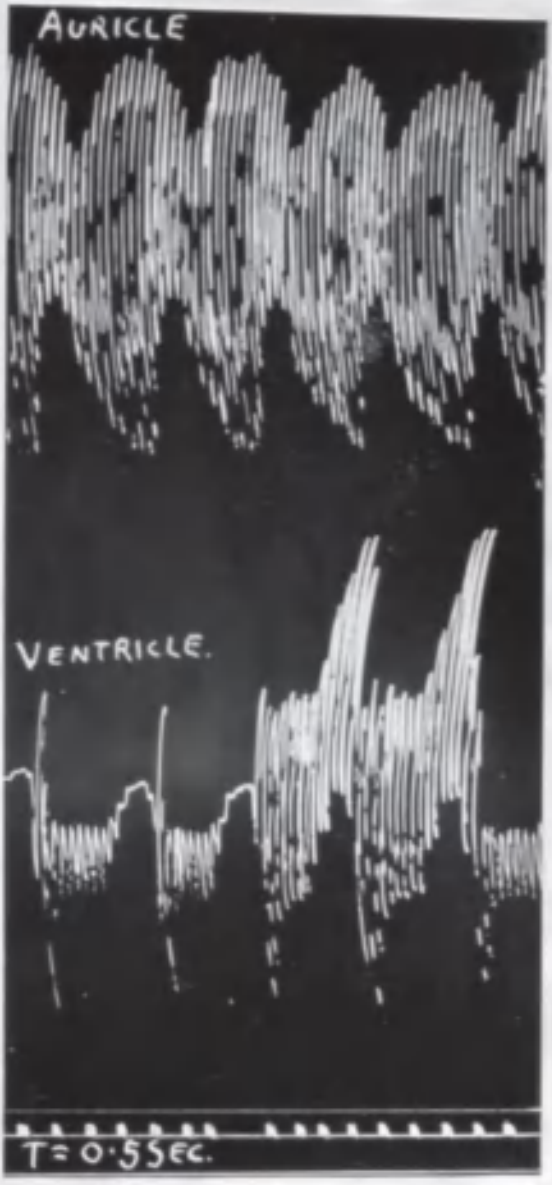

FIG. I4. (contd).

Showing ventricular intermittent tachycardia.

4 pm. Continued intravenous administration of Crataegus resulted in a gradual development of contracture of the auricle, the excursion falling to 41 mm. while the ventricle remained at 31 mm. at a heart rate of 138 per minute.

4.30 pm. Continued administration resulted in marked contracture of the auricle, with developing fibrillation and loss of the power to expel the blood into the ventricle. At this time the ventricular excursion remained at 25 mm. to an auricular excursion of 4 mm. at a heart rate of 108 per minute. With the development of auricular fibrillation marked irregularities in the degree, force and rate of ventricular contraction occurred.

4.40 pm. The auricle is fibrillating feebly. There is partial heart block, rapidly becoming complete so that the ventricle develops its own rhythm at a rate of 55 per minute, which gradually ceases from the increase of systolic contracture. Arrest is finally in a mid-systolic position. The auricle fibrillates for a short time after the ventricle has ceased. Ventricular fibrillation is seen at the end. These points are illustrated by fig. 14.

The action of Crataegus oxyacantha on the mammalian heart is thus similar to that of Digitalis. The action may be divided into two phases, a preliminary phase of inhibition, and a later phase of heart block, cardiac irregularity and effects direct on the muscle.

The first phase is removed by atropine, while if atropine be given before the Crataegus there is little effect on the heart, other than an increase in the systolic movement of the recording lever. The effect of Crataegus on the ventricle is thus compounded of two factors, the augmented inhibition and the greater strength of the systolic contraction. The first slows the rhythm and often increases the relaxation; the second increases the extent of the contraction and tends to limit the relaxation. In the auricle the same factors are in action; the rhythm is slowed in the same ratio as the ventricle, and this seems to be almost wholly due to the increased and prolonged diastolic pause, little change occurring in the duration of the active phase. The strength of the contraction is at first increased and diastolic relaxation is often more marked.

The two auricles beat together and the two ventricles; the pause between the contraction of the auricles and that of the ventricles is lengthened owing to inhibitory action.

The slowing of the heart in the early stages is due to changes in the pace maker which is reduced in activity by the inhibitory mechanism.

This early phase, marked by pace maker slowing from the inhibition and augmented contractility from direct action on the heart muscle, may gradually pass into the second phase of increased spontaneity, especially when small doses have been injected and the action is slowly developed.

But if a large dose of Crataegus is injected intravenously marked inhibition occurs at once. The auricle dilates immediately and may cease for some time to beat, later recovering irregular and slight contractions which gradually recover and become more regular and vigorous as tone is restored to the chamber muscle. The auriculo-ventricular rhythm is usually maintained throughout except for the period of total inhibition and standatill of the auricles, but frequently spontaneous ectopic systoles of ventricular origin, and again ventricular intermissions, are seen for a short time. Recovery is rapid and spontaneous.

The ventricular rate is reduced, the rhythm irregular and the beats forceful.

This stage or phase of excessive inhibition is only seen under large intravenous doses before atropine or with the vagi intact and is due to vagal inhibition. After atropine such a dose will cause diastolic inhibition of both auricle and ventricle, in a short time recovering to give an increased rate and force of beating; this latter action seems to be a peripheral nervous one, the drug acting on the vagal terminations in the heart. See fig. 14

This point has been much debated with regard to Digitalis ; the final conclusion of Ackermann (1873) was to the effect that a similar peripheral action could occur with Digitalis but that the chief action was on the medulla oblongata.

After atropine the rate of the heart under Crataegus is increased slightly above its normal rate (see protocol) and this resembles the action in the isolated perfused mammalian heart under Digitalis, for as the rhythm remains normal it is not due to increased spontaneity as is seen during the second stage of action of the drug.

The changes in contractility in the heart occur in the isolated heart, after atropinisation of the heart in situ, or after vagal section, and are thus due to direct action on the heart.

If the above procedures be carried out in order to remove central vagal inhibition, there is found to be an increase in the force and degree of systolic contraction of both ventricles after intravenous administration of Crataegus, while the degree of diastolic relaxation is unaffected; but these features are only seen following upon the preliminary phase of total diastolic inhibition due to peripheral vagal stimulation if the dose be large.

The second or toxic stage of the action of Crataegus on the intact mammalian heart is marked by the great increase in the tendency to spontaneous beats in different parts of the heart, which may lead to the development of ectopic rhythms and ends in fibrillation and the rapidly developing difficulty in the conduction of impulses between the auricle and the ventricle.

These changes in conduction and spontaneity are

both due to direct action on the heart muscle primarily, as they occur in the heart under atropine.

Heart block from direct action on the fibres of the bundle of His is only very slowly developed under Crataegus and is but rarely seen in a marked degree. Fig. 13 illustrates the development of complete block with an idio-ventricular rate of contraction.

The rhythm of the two auricles and the two ventricles is at this stage the same but the auriculo-ventricular rhythm is altered. Further action of Crataegus takes the form of increasing Contracture of auricle and ventricle, the excursion of the auricular lever lessening both in systolic and diastolic variation and tending towards a mid-point while the diastolic relaxation of the ventricular lever is lessened. Further exhibition of the drug tends to cause the auricle to pass rapidly into a state of fibrillation while the ventricle is markedly irregular and may reveal series of weak beats, or long diastloic pauses. Ultimately ventricular fibrillation supervenes and the heart stops in diastole, but not in a position of gross dilatation such as would occur in a heart arrested by vagal inhibition, since the direct action of the drug on the heart muscle has induced a degree of contracture.

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ACTION ON THE PERFUSED MAMMALIAN HEART.

The preparation used was a modification of Gunn's cannula as used for routine class work. It has no striking features. The heart is perfused with Ringer-Locke solution at a suitable temperature (38°C) via the aorta. Infusion of Crataegus oxyacantha was used, diluted with saline and perfused through the whole cannula at the same rate, pressure, and temperature.

The isolated perfused mammalian heart reacts to Crataegus in a manner similar to the frog heart.

Protocol: 25/7/38.

Rabbit. Female. Isolated heart perfused with Ringer-Locke at 38°C.

2 pm. Rabbit killed by a sharp blow to the nape of the neck, heart excised and plunged into ice cold Ringer-Locke solution and massaged to expel blood; heart then attached to Gunn's cannula perfusing oxygenated Ringer-Locke at 38°C and the tip of the ventricle fixed to a hook and tied down. Hooks attached to the auricle and ventricle then led to recording levers.

2.10 pm. Record begun.

Heart markedly irregular with typical record of auricular extra-systoles at a rate of 168 beats per minute. The ectopic beats arising in the auricle are quite irregular in their appearance. The excursion of the levers, taken as an indication of the degree of systole is, respectively, for the auricle 5 mm. and for the ventricle 14 mm.

2.11 pm. The perfusion of 50% fresh infusion of Crataegus oxyacantha rendered isotonic to Ringer = Locke solution results in the disappearance of the ectopic beats and the production of a strong regular systole of both auricle and ventricle of, respectively 6 mm. and 28 mm. excursion; an increase in the order of 100% in the ventricular excursion. The rate of contraction was 122 per minute and the rhythm regular.

2.25 pm. This condition of augmented and regular beats gave way gradually to a considerable degree of bigeminy of the pulse, some slight degree of which had been visible earlier in the record. The contractions of auricle and ventricle were now arrhythmic, giving rise to periodic variations in the trace. This is due to partial block. The auricle is relaxing, and tending towards diastole.

2.27 pm. The phase of periodic variations has passed off and the whole heart is now regular in rhythm, beating at a rate of 70 per minute. The beats are slow and strong, the excursion of the auricular lever 4 mm. and the ventricular lever 22 mm.

2.34 pm. The auricle is beating feebly and is diastolic in position. Heart block develops and in turn 1/5, 1/4, 1/3, and 1/2 block are seen.

2.43 pm. The auricle is fibrillating violently while the ventricle continues its steady rhythm of 60 per minute, and shortly the auricle ceases to record.

2.45 pm. The ventricle has developed a bigeminous

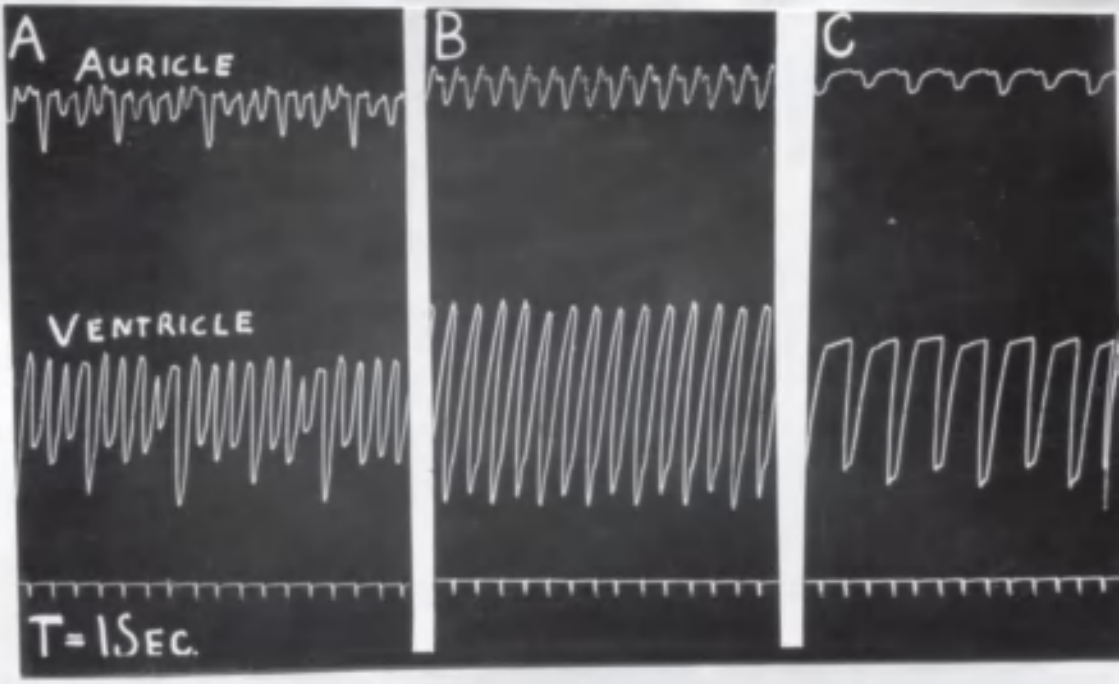

FIG. 13. Isolated Perfused Rabbit Heart.

Ringer-Locke 38 C. Effect of Crataegus.

A. Normal. Heart irregular.

B. After 50% Infusion of Crataegus - heart regular.

C. Marked slowing.

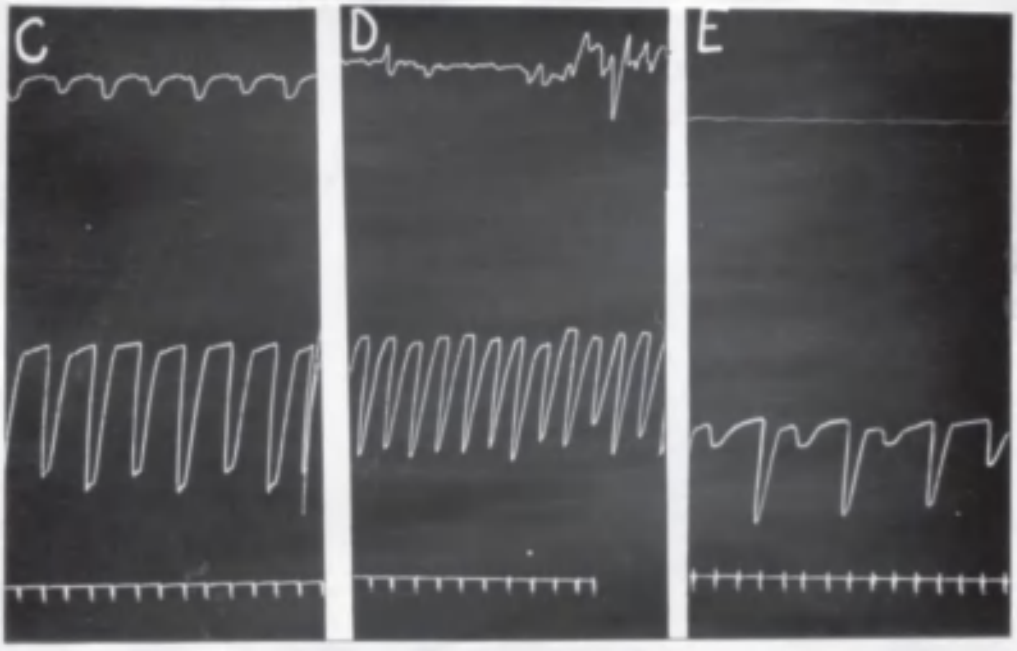

FIG. I3. (contd.).

D. Auricular fibrillation.

E. Heart block. Idio-ventricular rhythm.

rhythm which from its regularity would seem to be pulsus alternans, and shortly ceases in semi-systole.

These points can be seen in fig. № 13.

The significant points about such an experiment are several in number.

Firstly the removal by Crataegus Oxyacantha of the ectopic rhythm which is frequently a feature of the isolated perfused mammalian heart.

Secondly the early introduction and marked development of heart block, which is but little seen in the intact animal.

Thirdly the final arrest in auricular diastole and ventricular semi-diastole in contrast to the systolic effect of Digitalis.

Fourthly the fact that the early effects may be to some extent reversed by perfusion with saline, as in the case of the frog. This is only so in the early stages.

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THE ELECTROCARDIOGRAM.

The direct myocardiographic tracings of the intact and isolated mammalian heart were now correlated with the electrocardiographic records obtained on injection of Crataegus oxyacantha intravenously.

Protocol. 3/9/38.

Female cat, weight 3.2 kilos.

2. pm. Nembutal gr.

2.45 pm. Light ether anaesthesia completed. Right jugular vein exposed . Cat stretched on its back on insulated table and cotton wool plugs attached to the input leads of the electrocardiograph: these were inserted into the mouth and vagina , giving a long axis lead.

3. pm. Records of the normal heart taken.

3.5 pm. Injected 3 cc. tincture of Crataegus in 3 cc. saline.

3.8 pm. Records taken.

3.15 pm. Injected 3 cc. tincture of Crataegus in 3 cc. saline.

3.20 pm. Records taken.

3.25 pm. Injected 3.cc. tincture of Crataegus in 3 cc. saline. Death occurred.

3.30 pm. Female cat, weight 3 kilos, previously anaesthetised in the same way and similarly prepared, now arranged with the same input leads.

3.40 Records taken.

3.45 pm. Injected Diginutin (B.W. &Co) 1 cc. in saline

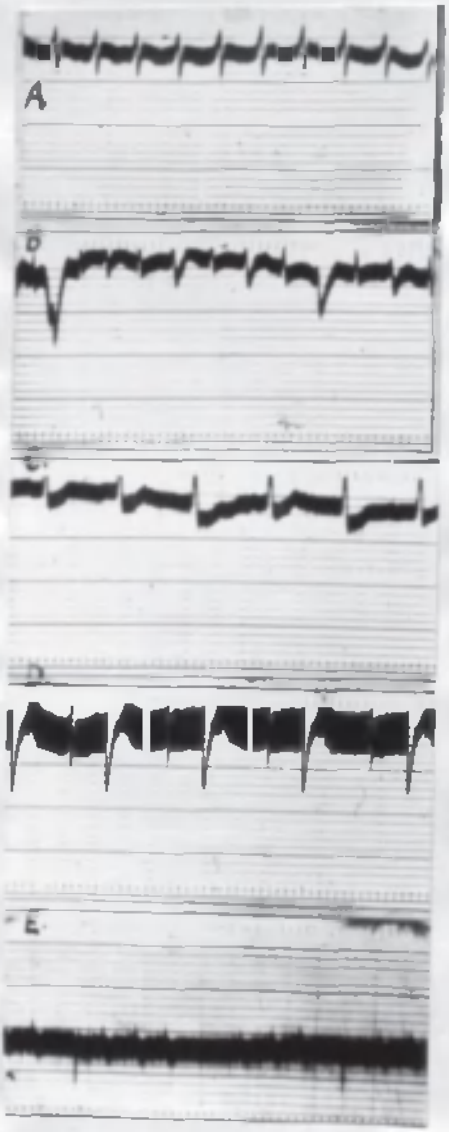

FIG. I5. Cat, female, 3.2 kilos weight.

Electrocardiogram - long lead.

A. Normal.

B. Effect of I unit of Digitalis.

C. Effect of 2 units.

D. Effect of 3 units.

E. Extra-systoles and auricular fibrillation.

3.50 pm. Records taken.

3.55 pm. Injected 1.cc. Diginutin in 4 cc. saline.

4. pm. Records repeated.

4.5 pm. Injected 1 cc. Diginutin in 4 cc. saline. Death.

The normal elctrocardiogram of cats and the effect on it of Digitoxin has been studied by Korth and Spung (1937) who report that intravenous doses of 0.1 to 0.3 mgm. Digitoxin per kilo o f cat flatten the T wave in a few hours without any physical change in the heart being apparent, such as necrosis: but that if the animals survive they show all the evidence of cardiac infarct with alteration of the T wave (inversion and abnormal place of origin of the S wave) and of the QRS complex, and that necrosis may then develop in the heart. In a cat of 4 kilos weight 0.2 mgm. Digitoxin per kilo induced in 8 days a slowing and strengthening of the pulse, increased PR interval, higher voltage of the R wave, and flattened T wave; the results obtained in the course of the present experiments with Digitalis are shown in fig. 15.

The normal cat electrocardiogram taken with these leads shows a heart rate of 220 beats per minute while the complex has a - ve. P and T wave and an upright R wave, - ve. S wave of small voltage, giving a familiar type of QRS complex with half on either side of the isoelectric line.

PQ = 0.068 sec: QS = 0.03 sec: RT = 0.1 sec.

Following upon 1 unit of Digitalis intravenously

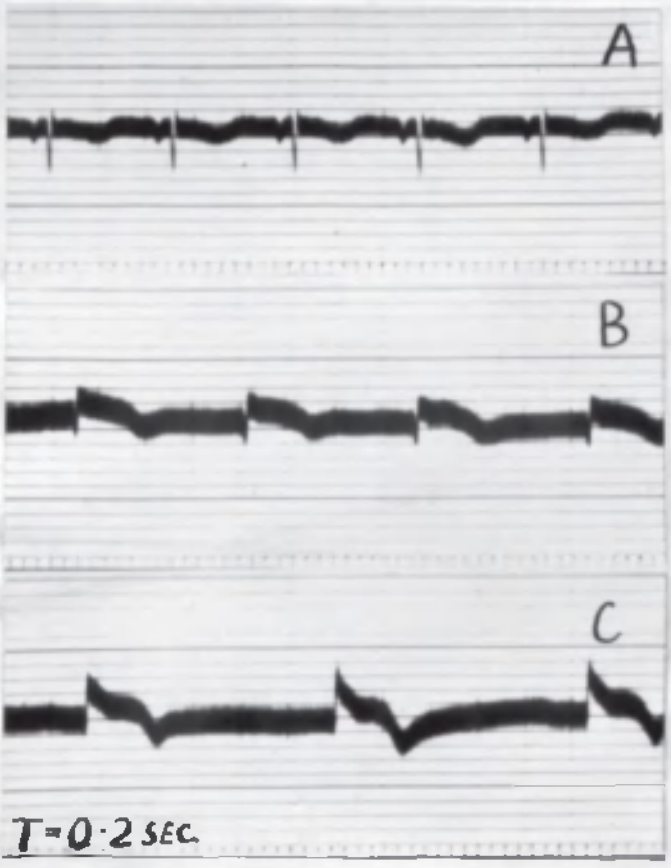

FIG. 16. Cat, female, 3.4 kilos weight.

Electrocardiogram - long lead.

A. Normal.

B. Effect of 3 cc. of Tincture of Crataegus.

C. Effect of 6 cc. of Crataegus.

the heart rate changes in a few minutes to 260 beats per minute with some irregularity of rhythm and the appearance of extra-systoles of various types, while the QRS complex tends to be simpler in form and entirely positive.

A further dose of one unit of Digitalis reduces the rate to 120 beats per minute, QRS is inverted and T flattened while P has lost its simple form and become more marked. The rhythm is not perfectly regular.

PQ = 0.14 sec.; QS = 0.05 sec.; RT = 0.19 sec.

A further dose of one unit of Digitalis reduces the heart rate to 40 beats per minute which is soon doubled by the appearance of coupled beats, every second beat being an extra-systole.

The P wave is again altered in shape and much strengthened. The QRS complex is restored to its positive form and the voltage increased by 50%, while the T wave is absent. The rhythm is irregular.

PQ = 0.17 sec.; QS = 0.07 sec.; RT = 0.20 sec.

These effects are unlike those obtained by Korth and Spung, and while the unusual features of the normal electrocardiogram may be explained by the unusual leads chosen, the varied inversions are noteworthy.

The effect of Crataegus oxyacantha on the cat electrocardiogram are seen in fig. 16.

The normal cat electrocardiogram with these leads is substantially as before.

The heart rate to begin with was 120 beats per minute, P wave distinct and - ve., QRS + ve. and T distinct and - ve.

PQ = 0.03 sec.; QS = 0.015 sec.; RT = 0.20 sec.

The effect of 3 cc. of tincture of Crataegus was at first to leave the rate unaffected at 120 beats per minute, while increasing the voltage of all components of the complex. Subsequently the pulse is regular at a rate of 80 beats per minute, P is absent, QRS biphasic, T accentuated and inverted as before and comes off the S wave in a peculiar manner.

Further 3 cc. of tincture of Crataegus given intravenously lead to the continuence of a heart rate of 80 beats per minute, with absent P wave (idio-ventricular beats) and the following intervals and time relations:-

QS = 0.01 sec.; RT = 0.30 sec.

This condition develops until the form of the T wave is much altered and rendered positive i.e. a true inversion of T occurs.

Further dosage with 3 cc. of tincture of Crataegus maintains the rhythm at 80 beats per minute but the voltage of QRS is increased, R is positive i.e. a true inversion of R has occurred, and T has become altered and separated from S which is now prominent, due to the great increase in the time taken by the QRS complex. T is restored to its original negativity.

QS = 0.09 sec.; RT = 0.30 sec.

A final intravenous dose of 3 cc. tincture of

Crataegus reduces the heart rate to 60 beats per minute with absent P wave, QRS + ve., QRS increased and S prominent, T inverted and prominent i.e. - ve, and the rhythm regular.

With Crataegus therefore, the auricle is early put out of action, conduction in the ventricle is greatly delayed, and the muscle is but little affected in that The T wave is not inverted or only temporarily so. With Digitalis fibrillation and heart block do not occur so readily, T is obliterated and P strengthened rather than abolished, and the rate of conduction in the ventricle but little affected.

It would seem therefore as if Crataegus acted more on the conducting system and less on the mode of contraction and wave form i.e. the muscle.

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Various methods of obtaining the normal electrocardiogram of the guinea pig have been suggested. The method employed was very simple. Six guinea pigs of the same sex and roughly 350 gm. weight were taken and the fore legs plucked of hair as far up the leg as possible. Each pig was given it a whiff of ether to render it sleepy and the fore legs placed in wells of saline in a wax block. Wires lead from the foot of each well of saline to the input leads of the electrocardiograph. It is essential to watch that saline does

not wet the animals' fur and create short circuits.

Pratt (1938) recommends that the guinea pig be slung up in a frame and the leads led off from the legs which are previously shaved and wrapped in saline soaked wool strands, the whole enclosed in a finger-stall with a rubber side tube for the strands cemented to it. This method was tried and proved impracticable as the animals were too restless.

Pratt gives a heart rate of 327 beats per minute for the guinea pig and these figures:-

P wave	= 0.0163 sec.	Systole	= 0.095 sec
PQ interval	= 0.036 sec.	Cardiac Cycle	= 0.183 sec
QRS complex	= 0.013 sec.	Ratio Systole/cycle	= 0.52
ST interval	=0.059 sec.	Frequency	= 327/min.
T wave	= 0.022 sec.		

Benedict (1915) gives a frequency of 267 per minute, Koenigsfeld and Oppenheimer (1922) give 290 per minute, Schott (1920) gives 290 per minute, and Buchanan (1910) 300 per minute, while Cornil et al. give 200 per minute. The sum of their opinion seems to be that the P wave is generally well marked but that the QRS complex is the most constant portion of the whole and that it is positive in sign. The T wave is negative in lead 1. positive in leads 2. and 3. Usually the rhythm is regular but sinus arrhythmia, ventricular extrasystoles, and dropped nodal beats are known.

Any appearance of a given animal is always reproduced subsequently if no interference with the action of the

heart has occurred.

In the present series the following pulse rates were recorded.

Fig No. 1. = 280 per minute.

" " 2. = 300 " "

" " 3. = 300 " "

" " 4. = 270 " "

" " 5. = 270 " "

" " 6. = 260 " "

It might be suggested that the high pulse rates recorded by Pratt were due to the restlessness and excitement of the animals which in our case rendered it impossible to make any records.

Cornil et al. (1933) remark that the P wave and T wave are positive and the QRS complex very prominent and upright.

They give the figures:-

PR	QRS	R	T	QT	Lead	Average.
0.065	0.041	0.025	0.081	0.14	1.	"
0.066	0.037	0.021	0.08	0.12	2.	"
0.09	0.05	0.03	0.10	0.15	1.	Maximum.
0.10	0.04	0.03	0.08	0.15	2.	"

The characteristics of the electrocardiograms obtained by us were as follows;-

Fig No.1.

" " 2.

" " 3. Lead 1. used throughout.

" " 4.

" " 5.

PR	QRS	R	T	QT
0.06 sec.	0.04 sec.	0.02 sec	0.03 sec	0.12 sec.
0.625 "	0.036 "	0.02 "	0.036 "	0.1 "
0.06 "	0.04 "	0.02 "	0.04 "	0.1 "
0.06 "	0.04 "	0.018"	0.025 "	0.09 "
0.06 "	0.03 "	0.008"	0.02 "	0.1 "
0.055 "	0.04 "	0.02 "	0.02 "	0.1 "

Following upon subcutaneous injection of tincture of Crataegus 0.5 cc. per 100 gm. pig daily for six weeks the form of the electrocardiogram is changed. The T wave and the P wave are absent, the QRS complex only 50% of its former voltage, the pulse rate 300 per minute and the QRS complex bifid in pigs no. 1 and 2, but in pig no. 3 the P and T waves are present and positive.

Diginutin (B.W.& Co) 0.1 unit per 100 gm. pig in all cases flattened the T wave, and in no case removed the P wave.

Pig No.	Lead.	Dosage for 6 wks.
1.	1.	Tr. Crat.1cc./100 gm.
2.	2.	Tr. Crat.1cc./100 gm.
3.	1.	Tr. Crat.1cc./100 gm.
4.	1.	Diginutin 0.1 cc./100 gm.
5.	1.	Diginutin 0.1 cc./100 gm.
6.	1.	Diginutin 0.1 cc/100 gm.

PR.

-

-

PR	QRS	R	Rate
-	0.025 sec.	0.020 sec	240/min.
-	0.033 "	0.02 "	180/min.
-	-	-	250/min.
0.025 sec.	0.03 "	0.01 "	270/min.
0.02 "	0.03 "	0.009 "	260/min.
0.02 "	0.03 "	0.01 "	270/min.

The effect of Crataegus on the conducting time in the bundle of His in the guinea pig heart is thus difficult to assess but the effect on the whole heart differs from that of Digitalis in that the action of the auricle is early affected, which bears out previous examinations of its cardiac action by other means.

Further dosage with Digitalis in the same amounts for 7 days produced considerable effects on the electrocardiogram of the guinea pigs no. 4,5,6 while no 1 and 2. had already succumbed to the toxic effects of Crataegus and no 3. was very seedy. Analysis of the electrocardiogram gives these figures:-

Fig No.	Dose/7 wks.	Lead.
3.	Tr.Crat.1cc/100gm.	1.
4.	Diginutin.0.1cc/100gm.	1.
5.	" " "	1.
6.	" " "	1.

PR	QRS	R	T	QT	Rate.
0.08 sec.	0.04 sec	0.02 sec.	-	-	280/min.
0.1 "	0.04 "	0.02 "	0.02 sec.	0.06 sec	180/min.
0.095 "	0.04 "	0.02 "	0.02 "	0.06 "	240/min.
0.1 "	0.0395"	0.02 "	0.02 "	0.06 "	220/min.

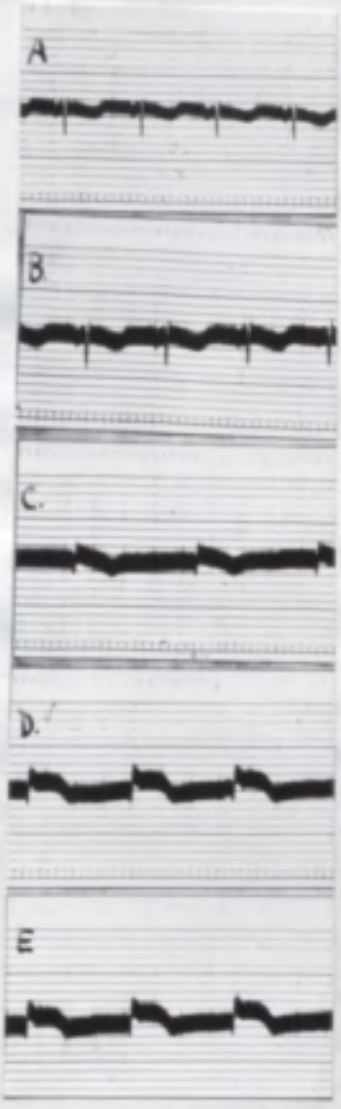

FIG. I7. Guinea-pig Electrocardiogram.

Effect of Crataegus, I cc. per day parenterally.

A. Normal.

B. On 7 th. day.

C. On I4 th. day.

D. On 2I st. day.

E. After 4 weeks.

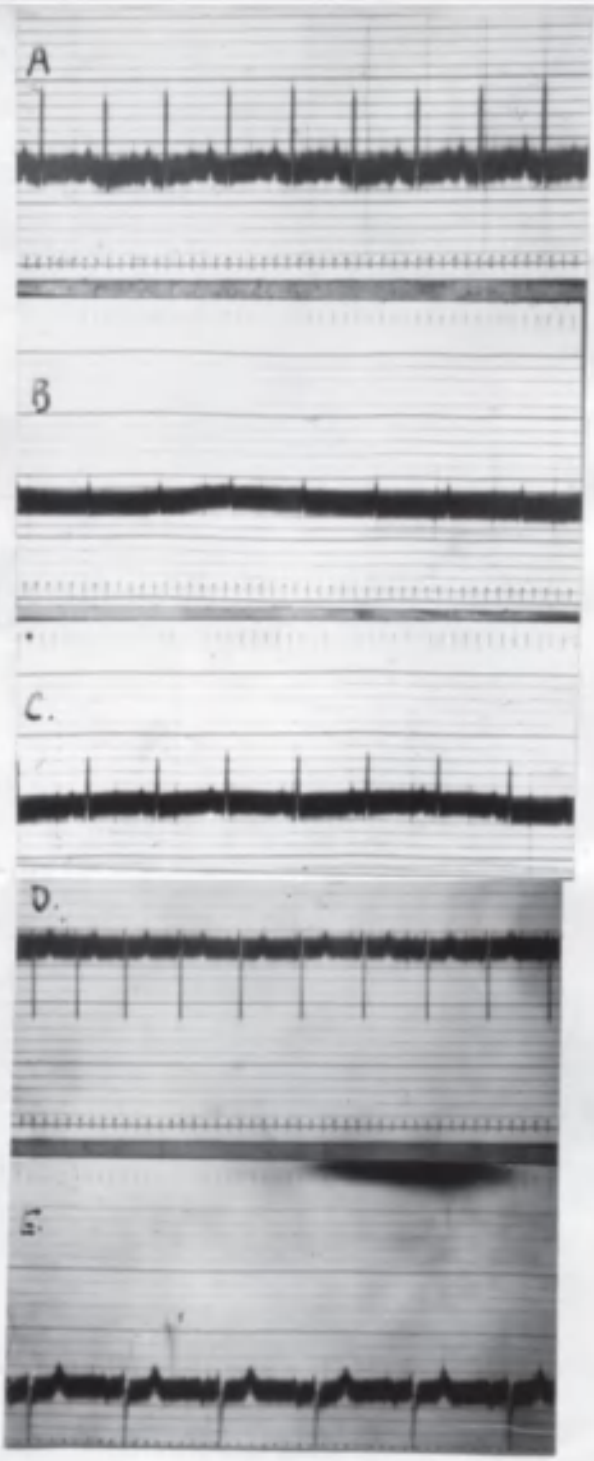

FIG. I7. (contd.). Digitalis.

A. Normal.

B. After I week.

C. After 2 weeks.

D. After 3 weeks.

E. After 4 weeks.

It is thus seen that no 3., which had received a sublethal dose of Crataegus presented the features of an absent T wave, an inverted P wave, a lengthened PR interval, and a positive QRS complex. Of the animals poisoned with Digitalis no. 4. showed an inverted P wave, positive T wave (small) and positive QRS complex, with a lengthened PR interval and shortened QT interval, while no. 5 and 6. reveal a positive P and T waves and lengthened PR interval.

These points are illustrated in fig. 17.

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PHARMACOLOGY. (contd.)

BLOOD PRESSURE.

In dealing with the pharmacology of the action of Crataegus oxyacantha on the blood pressure and the vessels mention only will be made of the effect of large doses given intravenously to experimental animals, or of perfusion experiments on isolated material, while all mention of the effect of therapeutic doses on the blood pressure and cardiovascular action of man will be considered later under the section on Therapeutics.

Tincture of Crataegus oxyacantha given intravenously in the cat in moderate doses, diluted with saline causes a temporary fall in blood pressure which may be of anaphylactic origin and is shortly compensated by a considerable rise in pressure, which, if the dose be large enough or the injection continuous, is followed by a progressive and marked fall in blood pressure. This fall may terminate fatally with the cessation of the heart beat, especially if the respiration be early inhibited. When artificial respiration is administered the terminal fall in blood pressure from a lethal dose is more gradual and prolonged.

Inhibition of the parasympathetic system by atropine, or section of the vagi enhances the rise in pressure which follows the preliminary fall.

Protocol. 5/5/38.

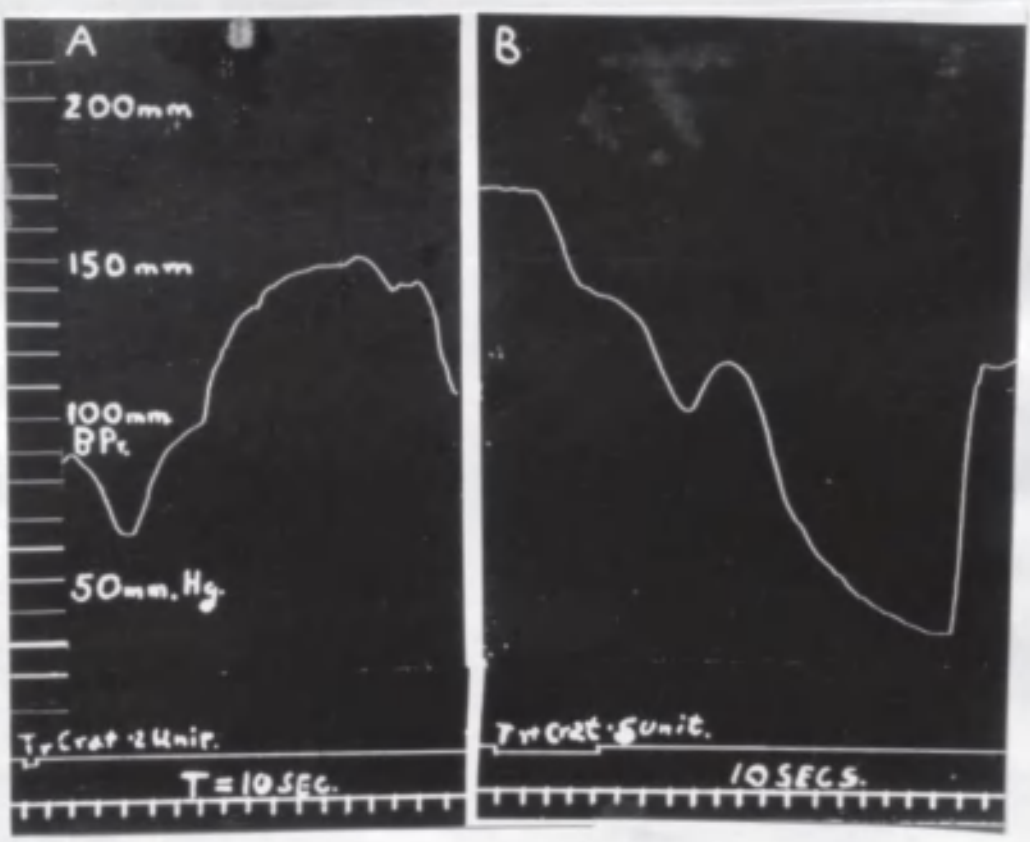


FIG. 18. Carotid Blood Pressure of Cat.

A. Anaphylactic fall and preliminary rise in pressure.

B. Prolonged fall in blood pressure.

Protocol. 5/5/38.

Cat ; male; 4.5 kilo. Nembutal 1.5 gr. 1 hr. previously; ether; Carotid blood pressure recorded.

3. pm. Atropine 1 mgm. intravenously: right vagus sectioned.

3.5 pm. Blood pressure stable at 100 mm. Hg.

0.2 unit of assayed tincture of Crataegus given by the right jugular vein.

Blood pressure fell to 70 mm. Hg. in 20 secs. and rose to 170 mm. Hg. in about 90 secs., respiration being inhibited completely.

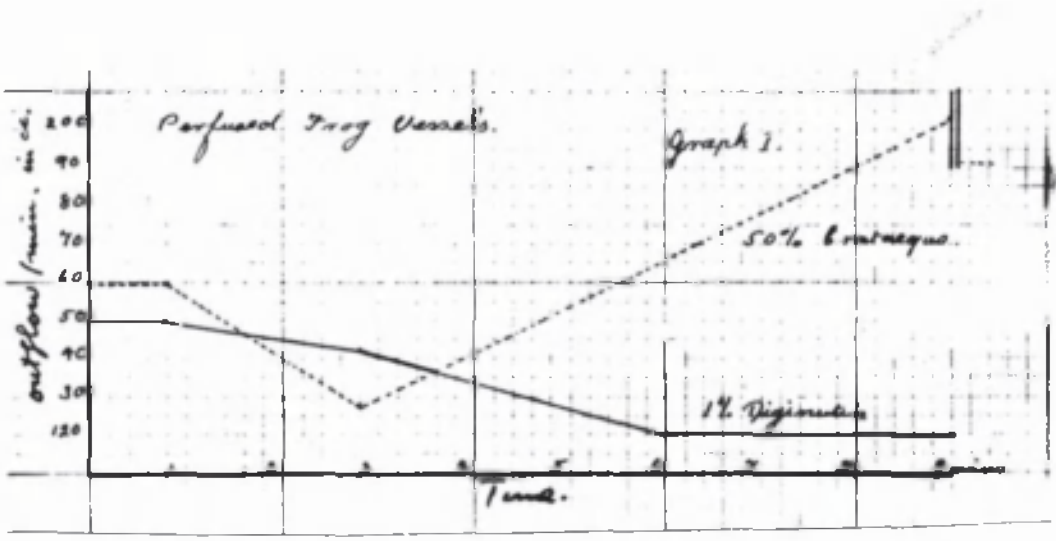
3.8 pm. Tincture of Crataegus 0.1 unit given by the right jugular vein in saline. Blood pressure fell from 170 mm. Hg. to 130 mm. Hg, whence it returned in 25 secs to 160 mm.Hg. and fell in 70 secs. to 60 mm. Hg., and recovered sharply as the inhibition passed from the heart in a short time.

The action on the blood pressure is thus compounded from the action on the heart and the action on the vessels; moderate doses lead, after a preliminary fall, to a sustained heightening of the blood pressure; larger doses lead to a prolonged depression of blood pressure.

These points may be seen in fig. 18.

ACTION ON THE BLOOD VESSELS.

The action of a total fluid extract of Crataegus oxyacantha on the systemic vessels of frogs and mammals and the coronary vessels of mammals has been investigated


Perfused Frog Vessels.
Graph 1.
outflow / min. in cc.
200
90
80
70
60
50
40
30
120
50% Ringer
1% Digitalis
Time.

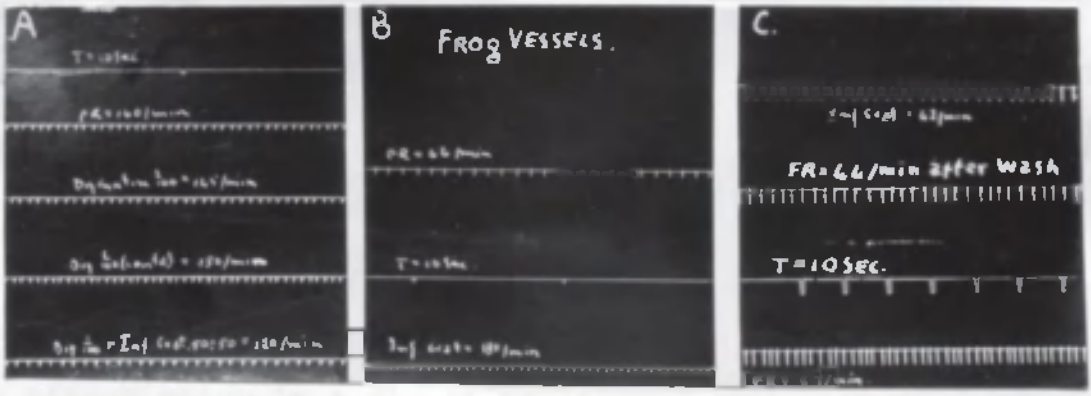


FIG. I9. Perfusion of the Frog's Vessels.

A. Effect of Digitalis - vaso-constrictor.

B. Effect of Crataegus - vaso- dilator.

C. Effect of small doses of Crataegus - vaso-constrictor.

and reported upon by Martini (1932). After the report of the findings of this series of experiments, given below, the results are compared with the findings of Martini in a short discussion.

(a) Perfusion of the Frog's Circulation.

The systemic circulation of the frog was perfused with frog-Ringer solution via the aorta, and the out-flowing fluid recorded by an electric drip recorder. Perfusion at constant pressure with fresh infusion of Crataegus at intervals and in small doses acts as a vaso-constrictor, but in larger doses or in continuous perfusion is a vaso-dilator to the extent of paralysing the vessels. There is no recovery under saline so that the effect which is a direct peripheral one on the vessel walls, is not reversible and is much stronger than that of 1% standard Digitalis tincture.

1% Digitalis is a vaso-constrictor: infusion of Crataegus is at first a vaso-constrictor but later a vaso-dilator.

This is shown in fig. 19 and graph. 1.

Protocol. 7/11/37.

(a) Frog, male ,weight 37 gm.

Perfused with frog-Ringer-Hart at 19.5 C and 30 cm. pressure

1. Perfusion rate steady at 150 drops per minute.

2. Perfused with 1% Diginutim (B.W.&Co.) in saline. Perfusion rate dropped to 143 drops per minute in 2 mins. and to 120 drops per minute in 5 mins.

(b) Male frog, weight 37 gm.

Perfused with frog-Ringer-Hart at 19.5°C and 30 cm. pressure.

1. Perfusion rate steady at 160 drops per min.

2. Perfused with 50% fresh infusion of Crataegus oxyacantha in saline.

Perfusion rate dropped to 128 drops per min. in 2 mins. and rose to 204 drops per minute in 10 minutes.

3. Perfused with Ringer-Hart at 19.5°C and 30 cm. pressure for 10 minutes.

Perfusion rate remained steady at 192 drops per minute.

(b) The Systemic Vessels of the Intact Mammal.

As attempts to perfuse the isolated limb of cat or rabbit failed, due partly to the fact that the precipitate of resins from the tincture or of proteins from the infusion when diluted with saline tended to block the finer vessels, these were abandoned and resort made to the photo-electric method of Hanzlik (1936). Photo-electric plethysmography has been developed by Hanzlik (1936) Hertzmann (1937), Bousmann (1934) and Matthes (1935). The principle is apparent from the short description and sketch of the apparatus appended.

Apparatus and Technique.

Light from a 12 volt 36 watt motorcar headlamp bulb passes through an Ilford micro 3. green filter held between two clean glass quarter plates, and through the shaven ear of a rabbit, along a cardboard cylindrical

lens hood of one inch diameter to a powerful photo-electric cell in its light tight box. Both ears of the rabbit are shaved and the animal tied on its back and the eyes shaded so that it does not struggle, while one ear is lightly attached to the end of the lens hood by adhesive plaster. The photo-electric cell is hooked up to a 76 volt H.T. battery via a 10,000 megohm resistance and thence to a mirror galvanometer (Pye) of 215 mm. deflection per micro-ampère at 1 metre, reading on a graduated scale.

Injections are made via the opposite ear vein. Any change in the vascularity of the ears is reflected in the amount of light transmitted to the cell and thus in the galvanometer reading. An increase in output amperage means vaso-constriction.

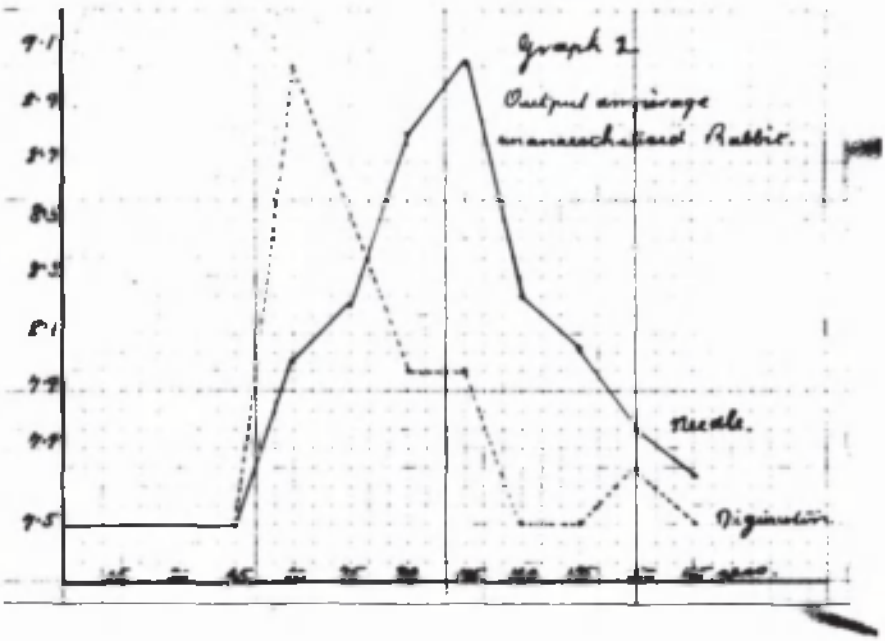
Protocol. 22/9/38:

Female rabbit, weight 3.2 kilo.

10 am. Shaved the previous day. Now prepared as indicated above, without anaesthesia, with the left ear at the filter and the right ear for injections.

(a) Injection of 1 cc. Saline.

Scale Reading.	Time.	Notes.
7.5	0 secs	Constant reading.
7.5	15 "	" "
7.5	30 "	" "
7.5	45 "	" "
8.0	60 "	Needling reflex.
8.2	75 "	Needle in Vein.
8.8	90 "	1cc. saline delivered.
9.0	105 "	Needle withdrawn.
8.3	120 "	-
8.0	135 "	-
7.8	150 "	-
7.6	165 "	-


Graph 2.
Output averages
of anaesthetized Rabbit.
Vascular.
Respiration.

(b) Injection of 0.25 unit of Digitalis in 1cc. Saline.

Scale Reading.	Time.	Notes.
7.5 divisions	0 secs.	Constant Reading.
7.5 "	15 "	" "
7.5 "	30 "	" "
7.5 "	45 "	" "
9.0 "	60 "	Needle In.
8.6 "	75 "	Needle Out.
8.0 "	90 "	-
8.0 "	105 "	-
7.5 "	120 "	-
7.5 "	135 "	-
7.6 "	150 "	-
7.5 "	165 "	-
7.3 "	180 "	-
7.2 "	195 "	-

The significance of these tables which are illustrated by graph no. 2 is that in the unanaesthetised animal there are vasomotor responses of a reflex nature in the one ear to handling, needling or injection of saline in the other ear. Digitalis injected into the ear vein of the unanaesthetised rabbit apparently causes vaso-constriction in the other ear but this may be purely reflex in nature.

Hanzlik (1936) in his experiments had his rabbits lightly anaesthetised with ether and the jugular vein cannulated.

Accordingly the experiments were repeated using an anaesthetic.

Protocol. 7/10/30.

Female rabbit, weight 1.7 kilo.

10 am. Nembutal 0.5 gr. in 3 cc. saline intramuscularly.

11 am. Preparation as before.

Moving the opposite ear, pinching it, and pricking it with a needle now had no effect on the vessels as

judged by the scale reading of the output amperage.

(c) Effect of Adrenaline by Ear Vein.

Scale Reading.	Time.	Notes.
34	0 secs.	Constant Reading.
34	15 "	" "
34	30 "	" "
34	45 "	" " (needling).
34.2	60 "	Adrenaline 150 γ by ear vein.
35	75 "	Needle out.
35.2	90 "	-
35	105 "	-
35	120 "	-

(d) Effect of Adrenalin by Jugular Vein.

Scale Reading.	Time.	Notes.
34.1	0 secs.	Constant Reading.
34.1	15 "	" "
34.1	30 "	" "
34.1	45 "	" "(needle in).
34.0	60 "	Adrenaline 150 γ by R. jugular vein.
33.9	75 "	Needle out.
33.5	90 "	-
33.5	105 "	-
33.6	120 "	-
33.8	135 "	-
33.7	150 "	-

Protocol. 7/10/38.

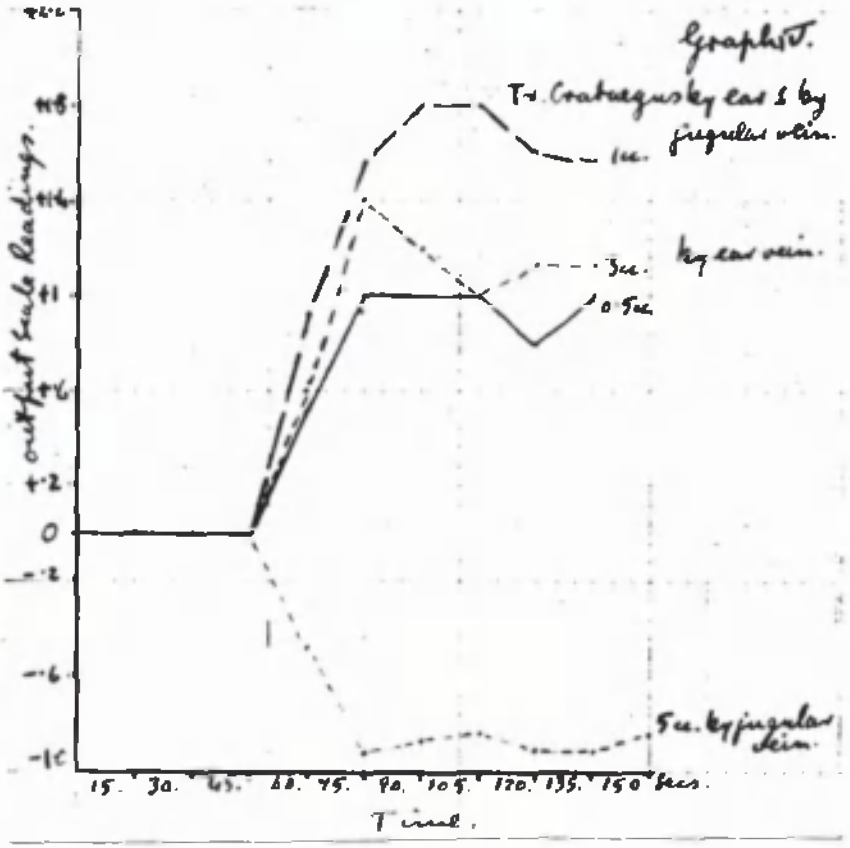
Female rabbit, weight 1.9 kilo.

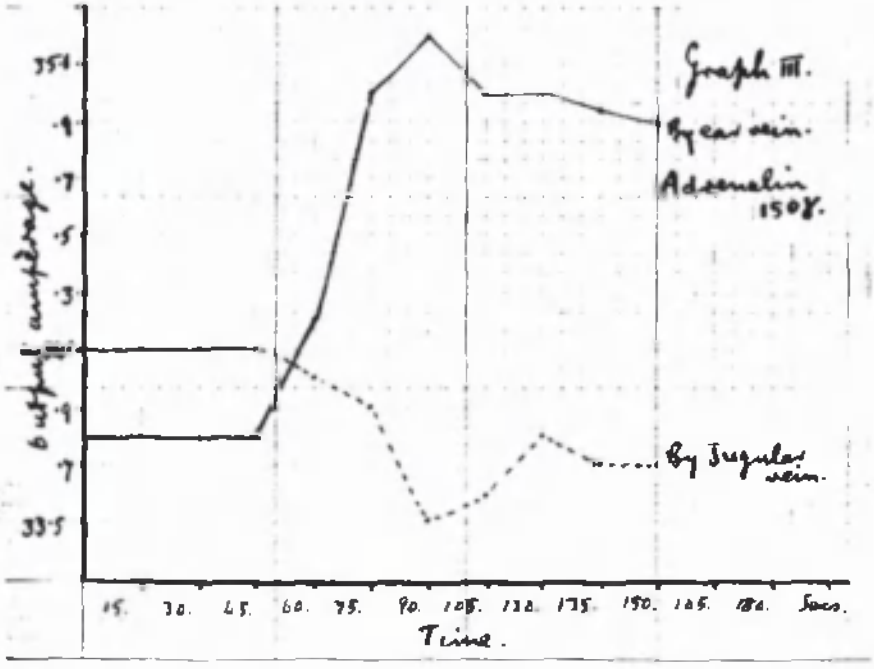
10 am. Nembutal 0.5 gr. in 2 cc. saline.

Preparation as above.

(e) The Effect of Tincture of Crataegus by Ear Vein.

Scale Readings.			Time.	Notes.
Dose. (a) 0.5cc.	(b) 1.0cc	(c) 3.0cc		
36	37	30	0 secs.	Constant Read
36	37	30	15 "	" "
36	37	30	30 "	" "
36	37	30	45 "	" "
36.5	37.9	30.6	60 "	Delivery.
37	38.5	31.4	75 "	Needle out.
37	38.8	31.2	90 "	-
37	38.8	31.0	105 "	-
36.8	38.8	31.2	120 "	-


Graph IV.
T. Crataegus by ear & by jugular vein.
1 Sec.
by ear vein.
3 Sec.
0 Sec.
5 sec. by jugular vein.
+ outflow Scale Readings.
+8
+4
+2
0
-2
-6
-10
15. 30. 45. 60. 75. 90. 105. 120. 135. 150 Secs.
Time.


Graph III.
By ear vein.
Adrenalin 1508.
By Jugular vein.
outflow amperage.
35·4
33·5
15. 30. 45. 60. 75. 90. 105. 120. 135. 150. 165. 180. Secs.
Time.

(f) The Effect of Tincture of Crataegus by Jugular Vein.

Scale Reading.	Time.	Notes.
35	0 secs.	Constant Readings.
35	15 "	" "
35	30 "	" "
35	45 "	" "
34.5	60 "	5cc.Tr. Crataegus by R. Jugular V.
34.0	75 "	Needle out.
34.1	90 "	-
34.0	105 "	-
34.2	120 "	-
34.1	135 "	-

The interpretation of these results is as follows:-

1. The unanaesthetised rabbit reacts to handling or needling of one ear by a reflex vaso-constriction of the other ear.

2. The anaesthetised rabbit reacts to the injection of Digitalis by vaso-constriction of the ear; to adrenalin given by ear with vaso-constriction; to adrenalin given by jugular vein with vaso-dilation. (Graph III)

3. The anaesthetised rabbit reacts to injection of Tincture of Crataegus oxyacantha by variable reactions. Up to 1/3 unit by ear causes vaso-constriction, over that amount by jugular vein causes vaso-dilation. (Graph IV).

The results show the variability of the method as various reflexes from ear to ear may be of great importance in determining the ultimate reaction. The results tend to show that Crataegus is a vaso-constrictor in the ear of the live rabbit, but if the animal is anaesthetised the vasomotor reactivity of the animal is much diminished and the sensitivity of the photo-electric cell, previously adequate, tends to become inadequate.

THE CORONARY ARTERIES.

The action of Digitalis and its allies on the coronary vessels of the heart has a special interest from a practical point of view and has been examined repeatedly. The general result of these experiments seems to be that the glucosides in poisonous doses constrict the coronary arteries like those of the rest of the body.

(a) The Isolated Coronary Arteries of the Sheep.

Method.

A young sheep was slaughtered and the heart and great vessels tied off, removed and plunged into ice cold saline. The first part of the right and left coronary arteries were excised and cut into a spiral strip which was mounted in a mammalian bowel-bath at 38°C in Tyrode-Bayliss solution and left for one hour to recover from initial spasm. The tension of the recordng lever was then adjusted and the bath oxygenated and drugs added as necessary.

Protocol. 1/2/38.

Right coronary artery of sheep prepared as above.

Left coronary artery of same sheep prepared also.

1. pm. Initial spasm passed off: base line of tonus recorded. Very slow drum geared down with Unicam one thousand reduction gear used.

1.5 pm. Fresh infusion of Crataegus oxyacantha 5% solution in Tyrode-Bayliss solution substituted. Vaso-constriction resulted and was maintained.

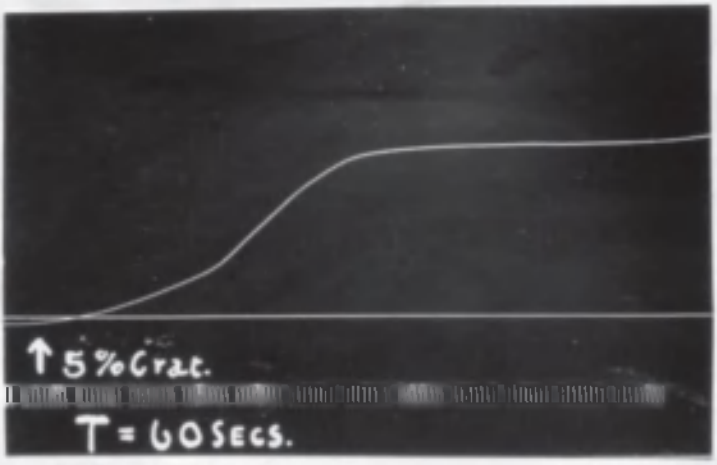


FIG. 20. Isolated Coronary Vessels of the Sheep.

Vaso - constrictor effect of 5% Crataegus.

1.15 pm. Adrenalin 100 γ added.

Vaso-dilation resulted.

1.30 pm. Left coronary artery similarly mounted.

Digitalis 1% standard tincture added.

Vaso-constrictor action.

1.40 pm. Adrenaline 100 γ added.

Vaso-dilation resulted.

See fig. 20

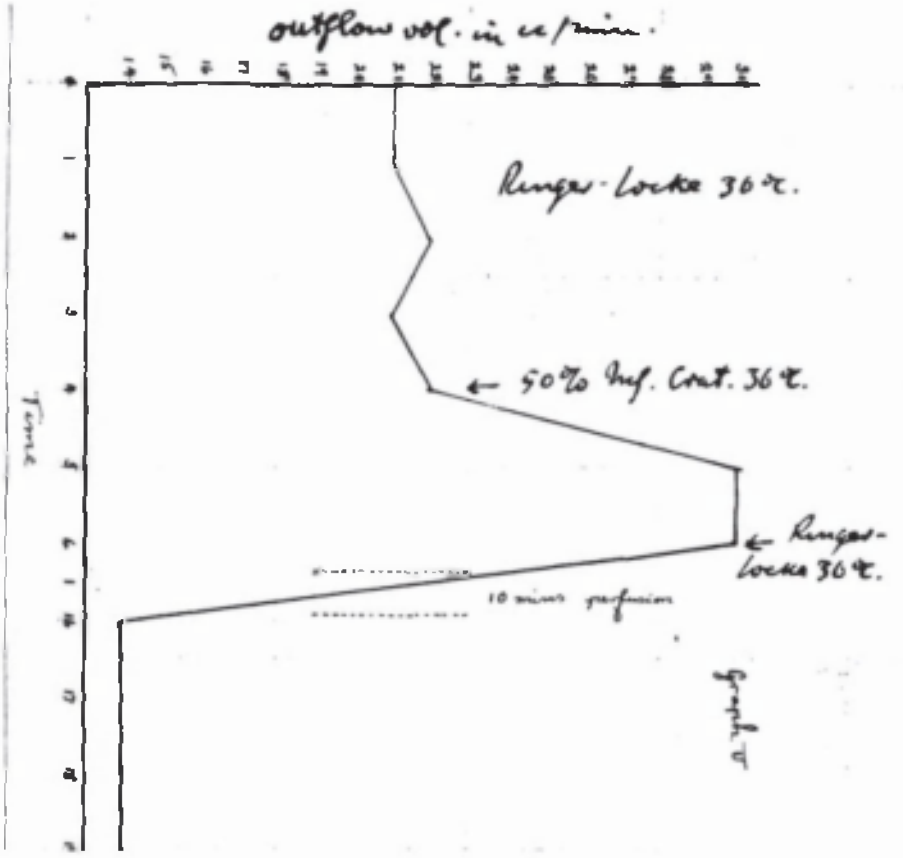
(b) The Perfused Isolated Rabbit Heart - Coronary Flow.

The preparation was the same as that already described for recording a myocardiograph from the isolated rabbit heart, the outflow per minute from the coronary circulation being measured, and in some cases recorded by means of an electric drop counter.

The following are a series of records of coronary outflow in cc. per minute.

Rabbit No.	1.	2.	3.	4.	Time in mins.
Ringer-	24.5 cc	30 cc	21 cc	25.6 cc.	1.
Locke 36C.	24.5 cc	28 cc	22 cc	25.4 cc.	2.
	24.5 cc	27 cc	21 cc	25.8 cc.	3.
	24.5 cc	28 cc	22 cc	25.7 cc.	4.
50% Inf.	28 cc	34 cc	30 cc	30 cc	5.
Crategus.	17 cc	26 cc	30 cc	30 cc	6.
Ringer-L.	13 cc	14 cc	14 cc	16 cc	16.
after 10	10.5 cc	14 cc	14 cc	15 cc	17.
mins. at	10.7 cc	14 cc	14 cc	15 cc	18.
36 C.	10.5 cc	14 cc	14 cc	15 cc	19.

This table is illustrated by graph. V


outflow vol. in cc/min.
Ringer-Locke 30°C.
← 50% Inf. Cont. 36°C.
← Ringer-Locke 30°C.
10 mins perfusion
Time
Graph II

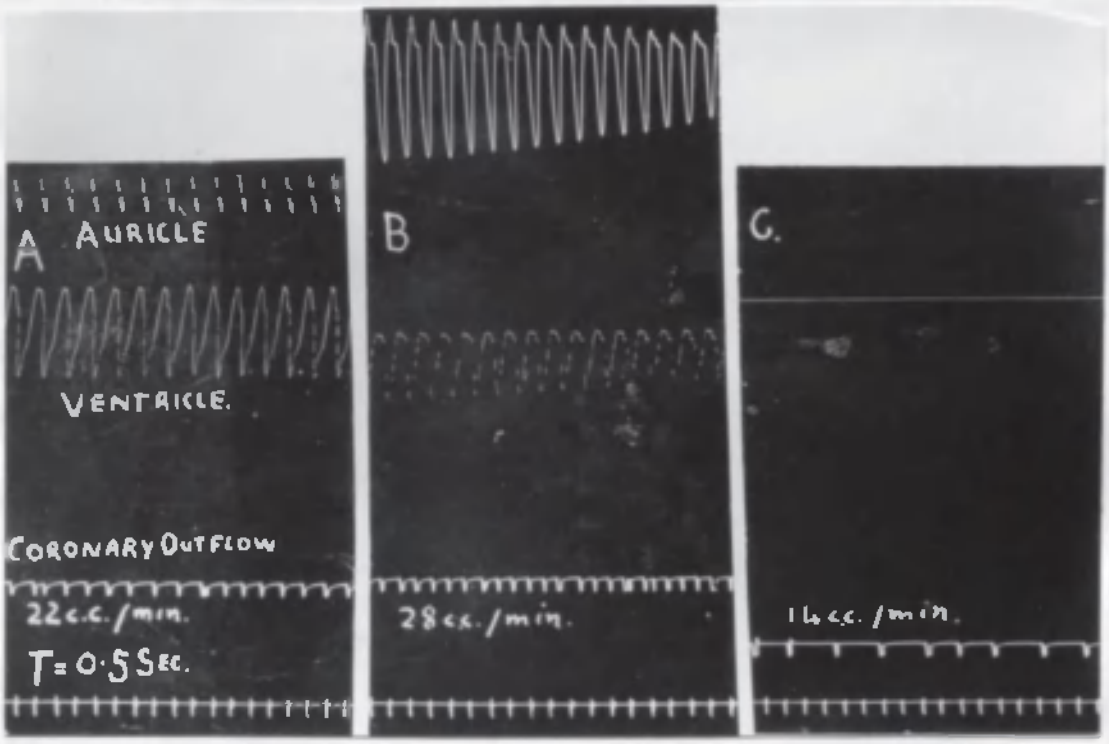


FIG. 2I. Coronary Outflow from Isolated Rabbit Heart - electric drop recorder.

A. Normal - 22.0 cc. per min.

B. 50% Inf. Crat. - vaso-dilator.

C. After 2 mins. - vaso-constrictor.

In the perfused isolated rabbit heart Crataegus oxyacantha thus acts as a preliminary dilator and then a constrictor. This constrictor action will presumably never develop with therapeutic doses, being counteracted by the increased force of action of the heart beat.

The preliminary vaso-dilation and the subsequent constriction as recorded by the drop counter is shown in fig. 21.

Martini (1932) obtained the following results in his study of the "haemodynamic actions of Crataegus oxyacantha".

1. No effect on the isolated frog heart. 50% fluid extract stops the heart gradually, but this is considered to be non-specific.
2. 1% fluid extract acts as a vaso-dilator of the perfused frog's vessels.
3. 1% fluid extract has no effect on the coronary vessels of the perfused isolated rabbit heart.
4. The carotid blood pressure of the dog falls profoundly and for a prolonged period, after intravenous injection of fluid extract, but this action is inhibited by atropine.

These results correspond to the results stated for the present series of investigations, but fail to bring out the dependence of the action on the dosage. The action on frog vessels, rabbit vessels, blood pressure of cat and coronary circulation are all reversible by suitable dosage. The effect on the coronary vessels is opposed to the action on the systemic vessels, unlike Digitalis which constricts both. The depressant effect

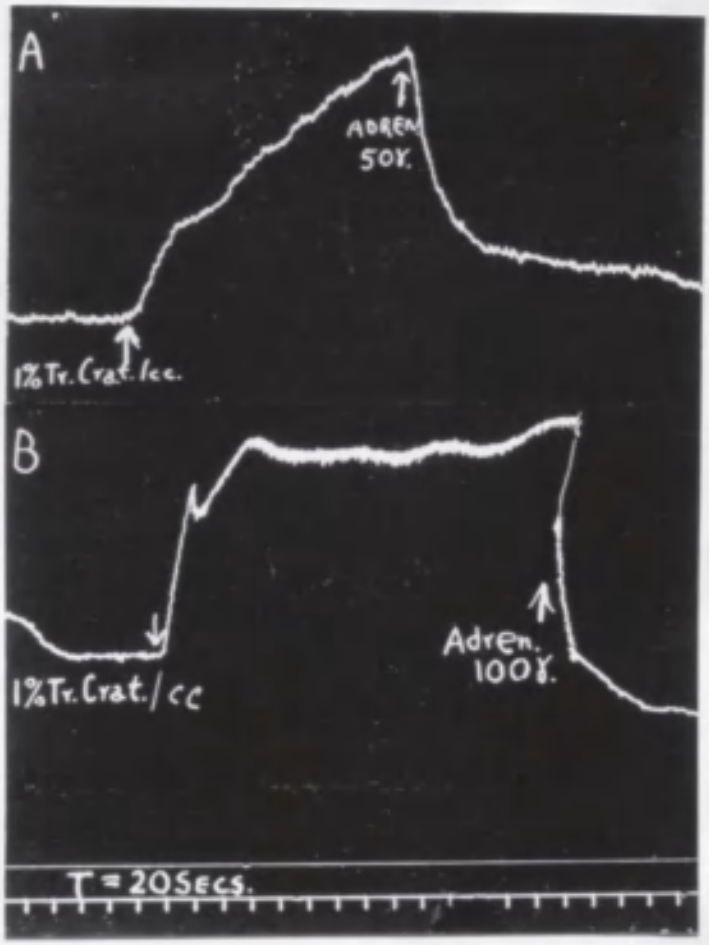


FIG. 22. Perfusion of the Isolated Bronchi and Pulmonary Vessels.

A. Broncho - constrictor effect of Crataegus.

B. Vaso - constrictor effect of Crataegus.

is thus opposed to its main action on the systemic vessels and accompanies its action on the coronary vessels.

It is difficult to comprehend why Crataegus should oppose Digitalis in its action on the systemic arteries and accompany it in its action on coronary and pulmonary vessels.

The action of Crataegus on the pulmonary vessels is illustrated in fig. 22.

--- --- ---

THE RESPIRATION and BRONCHI.

Digitalis and its allies tend to increase the pulmonary ventilation in animals, especially when given in large doses. The depth is more regularly increased than the rate: later the respiration becomes slower and deeper and may finally cease. In the rabbit a dose that will cause cessation of respiration is only 10% or so of the minimum lethal dose with artificial respiration.

Tincture of Crataegus oxyacantha given intravenously in the unanaesthetised rabbit, slowly by the ear vein, affects the respiration as follows:-

(a) Great increase in speed of respiration which becomes very shallow.

(b) Interpolation of occasional slow deep gasps.

(c) Slow deep respiration.

(d) Slow shallow respiration.

(e) Cessation of respiration.

In the anaesthetised animal, as for instance the cat prepared for recording the blood pressure with the respiratory movements recorded by a stethographic lever, a dose of Crataegus which scarcely affects the pressure, inhibits the respiratory movements entirely.

The fatal dose of Crataegus is therefore much less if artificial respiration be not supplied, and this point is of particular importance in assaying the tincture by continuous intravenous infusion in the cat.

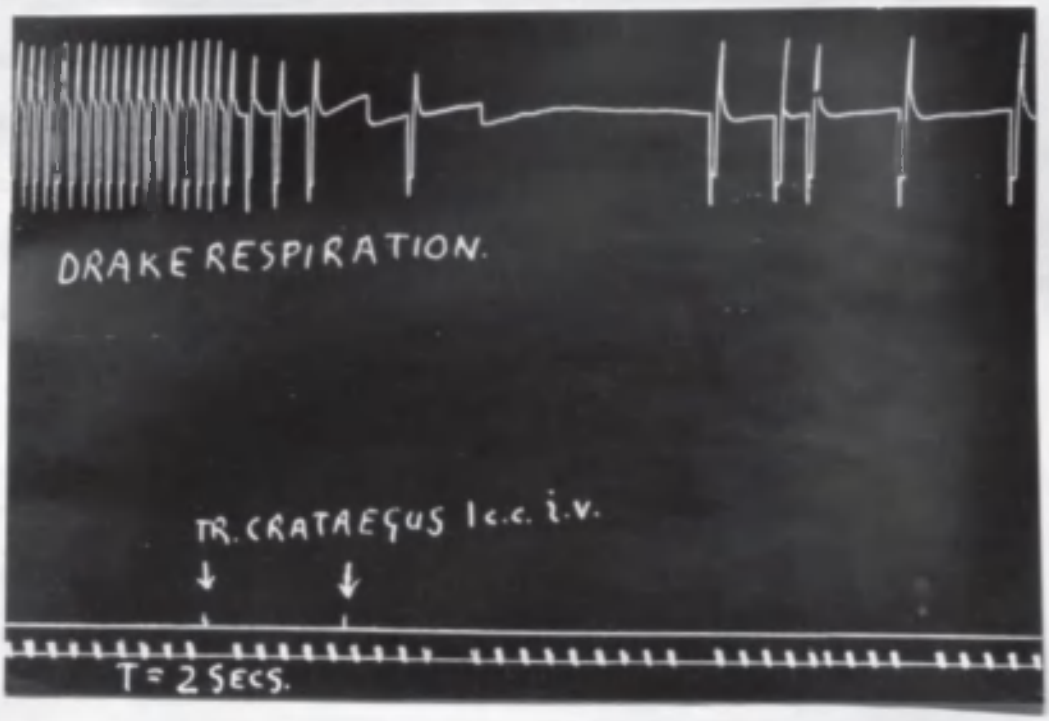


FIG. 24. Drake Respiration.

Inhibition of respiration by Crataegus.

This inhibition of respiration in the anaesthetised cat is [illegible]

In the drake the inhibition of respiration is not so marked, although it is obvious, as the following experiment shows.

Protocol. 9/11/38.

Drake, 2.4 kilo weight; nembutal 1 gr. intramuscularly.

10 am. Anaesthesia finished with light ether.

11 am. Tracheotomy performed, tube inserted and attached to a glass T tube containing pig gut inlet and outlet valve fittings. In this way respiration was possible while the changes in pressure could be recorded by a tambour attached to a side tube.

11.5 am. Corresponding points chosen.

11.6 am. Normal respiration recorded.

11.10 am. 2 cc. tincture of Crataegus in 2 cc saline given by jugular vein. Respiration became slow, apnoea followed quickly, and following upon a short series of slow and deep respirations, groups of three respirations with apnoeal pauses in between ensued and finally respiration ceased.

This inhibition of respiration is seen in fig. 24.

THE BRONCHI.

The method of perfusing the isolated guinea pig lung devised by Thornton (1932) was used. This has been described already.

Protocol. 30/11/38.

Male guinea pig, weight 380 gm.

11 am. Killed, thorax opened, and perfusing apparatus adjusted to give a constant rate of flow through the bronchial tree and the pulmonary circulation.

11.15 am. 1 cc. of 1% tincture of Crataegus oxyacantha injected into the pulmonary perfusion apparatus.

Immediate marked and sustained broncho-constriction resulted.

11.17 am. Adrenalin 100γ was injected into the arterial system.

The bronchi at once relaxed.

Crataegus oxyacantha is thus a broncho-constrictor. This is illustrated by fig. 22.

The similarity of action on bronchi and pulmonary vessels is unusual.

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EFFECT ON THE KIDNEY.

Their efficacy in increasing the urinary output and thus in reducing dropsy led to the introduction of Digitalis, Apocynum and others of the series, while Squills enjoyed a reputation as a diuretic for centuries. The action of the "digitaloid" series of drugs in dropsical patients would seem to be firstly a removal of the oedematous fluid by improvement of the circulation which results in hydraemia, and thus in diuresis.

The present series of experiments is a modification of the method of assay of the anti-diuretic principle of posterior pituitary as described by Burn (1936).

Method.

Eight rats of the same group were taken and weighed and isolated in two groups of four in circular metal cages with wire bottoming to them. These were put on large glass funnels with urine traps below, the funnels being arranged on a rack. The traps were designed to separate urine and faeces. The rats were put upon a standard rat diet and water ad lib. At four pm. the day before an experiment the food was removed and at eight am. on the day of the experiment the water was removed.

At 10 am. the rats are weighed and given 5 cc. of water per 100 gm. rat weight by stomach tube. This is a No. 3 gum elastic catheter moistened with glycerine and passed through a wooden rat gag in the mouth.

The method consisted of giving four rats a suitable

dosage of Digitalis, and four a control dose of saline; a few days later the groups were reversed and the process repeated.

The urine was collected and measured every fifteen minutes until diuresis was complete. The time from the mid-point of the injection period to the end of diuresis is the significant measure, rather than the total amount of urine excreted. After a suitable period the whole process was repeated, using tincture of Crataegus oxyacantha instead of Digitalis. It was thus possible to work on the same animals and so eliminate many variable factors, also determining by this method whether or not the known diuretic effect of Digitalis was apparent.

Protocol. 4/10/38.

Group A.			Group B.		
No.	Weight.	Water.	No.	Weight.	Water.
1.	253 gm.	12.5 cc.	1.	387 gm.	19 cc.
2.	187 "	9.0 "	2.	337 "	16.5 cc.
3.	227 "	11.25 "	3.	220 "	11 "
4.	210 "	10.5 "	4.	333 "	16.6 "
Total	877 "	43.25 "	Total	1275 "	63.1 "

The doses of water given in the table above were administered to the rats and the urine measured as it came off as shown in the next table.

Group A. 4 rats.			Group B. 4 rats.		
Water + 2cc. saline at 10.20			Water + Diginutin 0.25 unit + 1.75cc. saline at 10.42.5.		
Time.	Total Urine.	Vol. last 15 mins.	Time.	Total Urine.	Vol. 15 min
11.12	0.3 cc.	-	11.16	0.4 cc.	-
11.27	0.6 "	0.3 cc.	11.31	0.5 "	0.1 cc.
11.42	2.7 "	2.1 "	11.46	1.0 "	0.5 "
11.57	5.7 "	3.0 "	12.1	2.2 "	1.2 "
12.12	12 "	6.3 "	12.16	5.3 "	3.1 "
12.27	15.8 "	3.8 "	12.31	5.3 "	0.0 "
12.42	18.7 "	2.9 "	12.46	5.3 "	0.0 "
12.57	19.7 "	1.0 "			
1.12	21.8 "	2.1 "			
1.27	22.8 "	1.0 "			
1.42	23.5 "	0.7 "			
1.57	23.5 "	0.0 "			

Group A. Total Urine 23.2 cc. urine.

Half this plus the initial output is 11.6 cc. + 0.3 cc.

= 11.9 cc.

This was excreted between 11.57 and 12.12.

Point of maximum excretion = 12.11.2 mins.

Time to this point = 110.2 mins.

Group B. Total urine 4.9 cc. urine.

Half this plus the initial output is 2.45 cc. + 0.4 cc.

= 2.85 cc.

This was excreted between 12.1 and 12.16 mins.

Point of maximum excretion = 12.3 pm.

Time to this point = 80.5 mins.

Protocol 6/10/38.

Group A.			Group B.		
No.	Weight.	Water.	No.	Weight.	Water.
1.	212 gm.	10.5 cc.	1.	291 gm.	14.5 cc
2.	183 "	9.0 "	2.	294 "	14.6 "
3.	222 "	11.0 "	3.	219 "	11 "
4.	260 "	13.0 "	4.	322 "	16 "
Total.	877 "	43.5 "	Total.	1126 "	86.1 "

Group A. 4 rats.

Water + 0.25 unit Dig. + 1.75 cc. saline 10.5 am.

Time.	Total Urine.	Vol. 15 mins.
11 am	0.2 cc.	-
11.15	2.6 "	2.4 cc.
11.30	10.3 "	7.7 "
11.45	16.0 "	5.7 "
12.0	18.8 "	2.8 "
12.15	20.5 "	1.7 "
12.30	20.5 "	0.0 "
12.45	20.5 "	1.0 "
1.0	20.5 "	0.0 "
1.15	21.5 "	0.0 "

Group B. 4 rats.

Water + 2 cc. saline at 9.53 am.

Time.	Total Urine.	Vol. 15 mins.
10.40	2.1 cc.	-
10.55	4.5 "	2.4 cc.
11.10	8.5 "	4 "
11.25	16 "	7.5 "
11.40	18.2 "	2.2 "
11.55	23.1 "	4.9 "
12.10	31 "	7.9 "
12.25	31.2 "	0.2 "
12.40	32.6 "	1.4 "
12.55	34 "	1.4 "
1.10	34 "	0.0 "
1.25	34.5 "	0.5 "
1.40	34.5 "	0.0 "
1.55	34.5 "	0.0 "

Group A.

Total Urine excreted = 21.3 cc.

Half this plus initial urine = 10.65 cc. + 0.2 cc.

= 10.85 cc.

Excreted between 11.30 am. and 11.45 am.

Point of maximum excretion = 11.32 am.

Time to this point = 87 mins.

Group B.

Total Urine excreted = 32.4 cc.

Half this + initial urine = 16.2 cc. + 2.1 cc.

= 18.3 cc.

Excreted between 11.40 am. and 11.55 am.

Point of maximum excretion = 11.41 am.

Time to this point = 108 mins.

Thus we see from the first series of experiments that Digitalis reduces the total volume of urine excreted but also reduces the time taken to the point of maximum excretion and is thus a diuretic as to the time (which is the significant factor) but antidiuretic as to the volume of urine.

Protocol. 16/10/38.

Group A.			Group B.		
No.	Weight.	Water.	No.	Weight.	Water.
1.	176 gm.	8.9 cc.	1.	216 gm.	10.6 cc.
2.	206 "	10.2 "	2.	306 "	15.2 "
3.	214 "	10.6 "	3.	307 "	15.2 "
4.	240 "	12.0 "	4.	267 "	13.4 "
Total	836 "	51.7 "	Total	1094 "	64.4 "

It was presumed that ten days was a sufficient interval to allow of all the Digitalis, previously administered, being excreted, and the animals returning to normal.

Group A. 4 rats.			Group B. 4 rats.		
water + 2cc. saline 10.25			water + 2cc. Tr.Crataegus 10.40		
Time.	Total Urine.	Urine in last 15 min	Time.	Total Urine.	Urine in last 15 mins.
11.10	0.8 cc.	-	11.30	0.8 cc.	-
11.25	1.6 "	1.0 cc	11.45	3.7 "	2.9 cc.
11.40	7.0 "	5.4 "	12.0	7.0 "	3.3 "
11.55	14.9 "	7.9 "	12.15	7.5 "	0.5 "
12.10	21.0 "	6.1 "	12.30	14.2 "	6.7 "
12.25	24.5 "	3.5 "	12.45	15.2 "	1.0 "
12.40	24.5 "	0.0 "	1.0	17.7 "	2.5 "
12.55	25.5 "	1.0 "	1.15	22.0 "	4.3 "
1.10	25.5 "	0.0 "	1.30	22.0 "	0.0 "
1.25	25.5 "	0.0 "	1.45	25.0 "	3.0 "
			2.0	25.3 "	0.3 "
			2.15	26.5 "	1.2 "
			2.45	26.8 "	0.0 "
			3.0	26.8 "	0.0 "

Group A.

Total Urine excreted = 24.9 cc.

Half this plus initial urine = 12.45 cc. + 0.6 cc.

= 13.05 cc.

Excreted between 11.40 and 11.55 am.

Point of maximum excretion = 11.51

Time to this point = 86.5 mins.

Group B.

Total Urine excreted = 29.9 cc.

Half this plus initial urine = 13.0 cc. - 0.8 cc.

= 13.8 cc.

Excreted between 12.15 am. and 12.30 am.

Point of maximum excretion = 12.28 am.

Time to this point = 108 mins.

Group A.			Group B.		
No.	Weight.	Water.	No.	Weight.	Water.
1.	169. gm.	8.5 cc.	1.	207 gm.	10.3 cc.
2.	206 "	10.3 "	2.	249 "	12.5 "
3.	200 "	10.0 "	3.	302 "	15.0 "
4.	234 "	11.7 "	4.	292 "	14.6 "
Total.	809 "	40.5 "	Total.	1050 "	52.4 "

Group A. 4 rats.			Group B. 4 rats.		
Water + 2cc. Tr. Crat. 10.12			Water + 2cc. saline at 10.27		
Time.	Total Ur.	Urine 15 m.	Time.	Total Urine.	Urine last 15
11.13	0.4 cc.	-	11.24	0.9 cc.	-
11.28	1.0 "	0.6 cc.	11.39	2.1 "	1.2 cc.
11.43	6.9 "	5.9 "	11.54	4.4 "	2.3 "
11.58	7.6 "	0.7 "	12.9	9.5 "	5.1 "
12.13	9.0 "	1.4 "	12.24	12.5 "	3.0 "
12.28	11.7 "	2.7 "	12.39	17.0 "	4.5 "
12.43	11.7 "	0.0 "	12.54	20.0 "	3.0 "
12.58	13.0 "	1.3 "	1.9	23.0 "	3.0 "
1.13	13.0 "	0.0 "	1.24	23.0 "	0.0 "
1.28	17.4 "	4.4 "	1.39	23.2 "	0.2 "
1.43	17.5 "	0.1 "	1.54	23.2 "	0.0 "
1.58	17.5 "	0.0 "	2.9	23.2 "	0.0 "

Group A.

Total Urine excreted = 17.1 cc.

Half this plus initial urine = 8.55 cc. + 0.4 cc.

= 9.0 cc.

Point of maximum excretion = 12.13 am.

Time to this point = 120.5 mins.

Group B.

Total Urine excreted = 22.3 cc.

Half this plus initial urine = 11.15 cc + 0.9 cc.

= 12.0 cc.

Excreted between 12.9 am and 12.24 am.

Point of maximum excretion = 12.22 am.

Time to this point = 115 mins.

We thus see that Tincture of Crataegus oxyacantha injected subcutaneously in rats has an anti-diuretic effect in contr ast to Digitalis which has a diuretic effect.

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THE CENTRAL NERVOUS SYSTEM.

It is difficult to assess the effect of a drug on the central nervous system of animals. It is an old and confused controversy as to whether or not various effects of Digitalis are due to circulatory failure or a direct action on the central nervous system.

The frog injected with tincture of Crataegus shows immediate and marked irritation, presumably due to to the act of injection. It then settles down quietly if undisturbed. The breathing becomes fast and deep, and if the dose be large the muscles relax, the animal lies flaccid with gaping mouth and slow shallow breathing. At this time the heart is still beating quite vigorously, though slowly, so it is probable that these effects and the marked feebleness and slowness of reflex responses are due to a direct action on the nervous system.

In the cat and the drake emesis may be induced and is also frequently seen in anaesthetised animals following upon large intravenous dosage, while micturition, defaecation and even a degree of diarrhoea may be seen following upon such dosage. These latter effects may be simply due to relaxation of sphincters. Certainly Crataegus like Digitalis will induce emesis if given intravenously.

Given by ear vein to the rabbit, tincture of Crataegus oxyacantha induces the rapid changes in respiration already detailed under the study of the effects on the lung and repiration. It is also noticeable that some time

before cessation of the respiration occurs the neck muscles show marked tremor, the hair of the back stands on end, there are chewing movements of the jaws, and the conjunctival reflex is lost. Relaxation of the body, in particular the hind legs and musculature of the lower back occurs before cessation of the respiration.

These phenomena would seem to give some indication of a stimulant action on the medulla oblongata of the rabbit.

Neither the guinea pig or the rat seemed to be affected in any way by repeated subcutaneous injections of Crataegus, in so far as the central nervous system was concerned.

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UNSTRIATED MUSCLE.

Smooth muscle is thrown into a state of increased tone and movement by the "digitaloid" glucosides, requiring for this effect a greater concentration than affects the heart(Clark 1912). This action has also been observed in a few cases in the intact animal Gaisbock (1915).

(a) The Vessels.

Small doses cause constriction, larger doses dilate. The coronary vessels are dilated by small doses, constricted by large doses: the pulmonary vessels are constricted by small doses.

(b) The bronchi are constricted.

(c) The Bowel.

Alvarez (1919) studied the action of drugs on different parts of the bowel. According to Gaskell (1916) the muscle of the pyloric and ileo-colic sphincters probably has a different phylogenetic origin from that of the rest of the gut. Digitalis 0.5% solution in Ringer solution inhibits the action of the duodenum and jejunum and has a motor action on the middle ileum, lower ileum and colon of the rabbit. In this way the normal activity gradient of the bowel may be reversed and it was erroneously thought that this might be the causal factor in giving rise to emesis.

With many drugs small doses stimulate and large doses depress the action of the bowel.

1. Bowel in Vitro.

(a) Rabbit.

1. First part of Jejunum.

Infusion of Crataegus 0.5% has no effect, 1% is inhibitor, 5% is inhibitor. The inhibition affects the motor activity of the bowel wall but not the state of tonus of the muscle.

2. Middle Jejunum.

Infusion of Crataegus 3% is motor to the bowel musculature. Lesser concentrations were not tried.

3. Ileum.

Infusion of Crataegus 0.75% is motor to the ileum. 7% is inhibitor.

4. Colon.

Infusion of Crataegus 3% is motor to the colon.

These and other experiments on the effect of Infusion of Crataegus oxyacantha on the motor activity and state of tonus of the bowel musculature were carried out in a mammalian bowel bath apparatus containing 200 cc. of Tyrode-Bayliss solution at 38°C. Drugs were added by pipette after warming of the solutions. Aeration with a 4% CO2 mixture was maintained.

The general impression given is that small doses of Crataegus oxyacantha are motor to the isolated bowel suspended in oxygenated warmed Tyrode-Bayliss solution, while larger doses inhibit it: the motor gradient of the alimentary tract tends to be reversed by Crataegus.

These results, first obtained on the bowel of freshly killed rabbits, have been confirmed on the bowel of

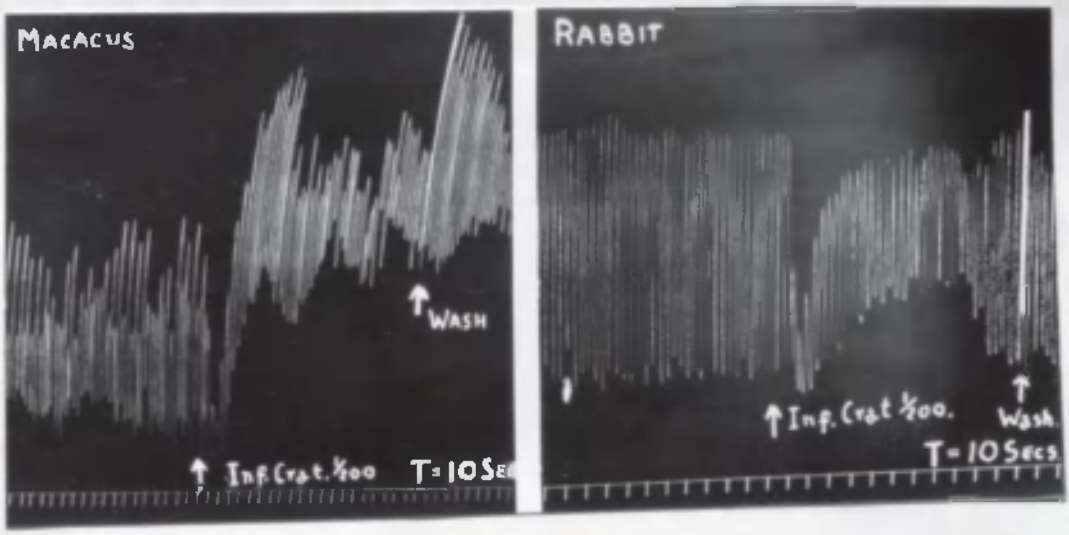

FIG. 25. Effect of Crataegus on Intestine in Vitro.

Macacus - Motor effect on Jejunum.

Rabbit - Inhibitor effect on Jejunum.

rat, guinea pig, cat, duck and monkey (Macacus).

Rat gut is weak in its movements and very slow in its peristaltic wave. The movements must be recorded on a very slow drum with high magnification.

Guinea pig gut is also weak and tends to be erratic in its movements but is much quicker in the rhythm of its peristalsis.

Cat gut is very stiff and records best when heavily loaded and is then quite satisfactory.

Duck gut is very weak and erratic in its movements and has a very poor peristaltic wave. It is difficult to record. There is some possibility of its autonomic innervation or reaction to drugs being different from that of mammals. (Graham 1938).

Monkey (Macacus) bowel gives strong rhythmic contractions but is easily affected by lack of oxygen or change of temperature.

The effect of Crataegus oxyacantha is inhibited by atropine and does not take place in Ca free Ringer solution but is otherwise similar to that of Digitalis. It can not be reversed by washing with saline.

These effects can be seen in fig. 25.

2. Bowel In Vivo.

The method of recording bowel movement in vivo is illustrated by the following experiment:-

Protocol. 13/5/38.

10 am. Female guinea pig, weight 400 gm.

Chloralose 20 mgm. In 20 cc. saline kept at 60°C until all

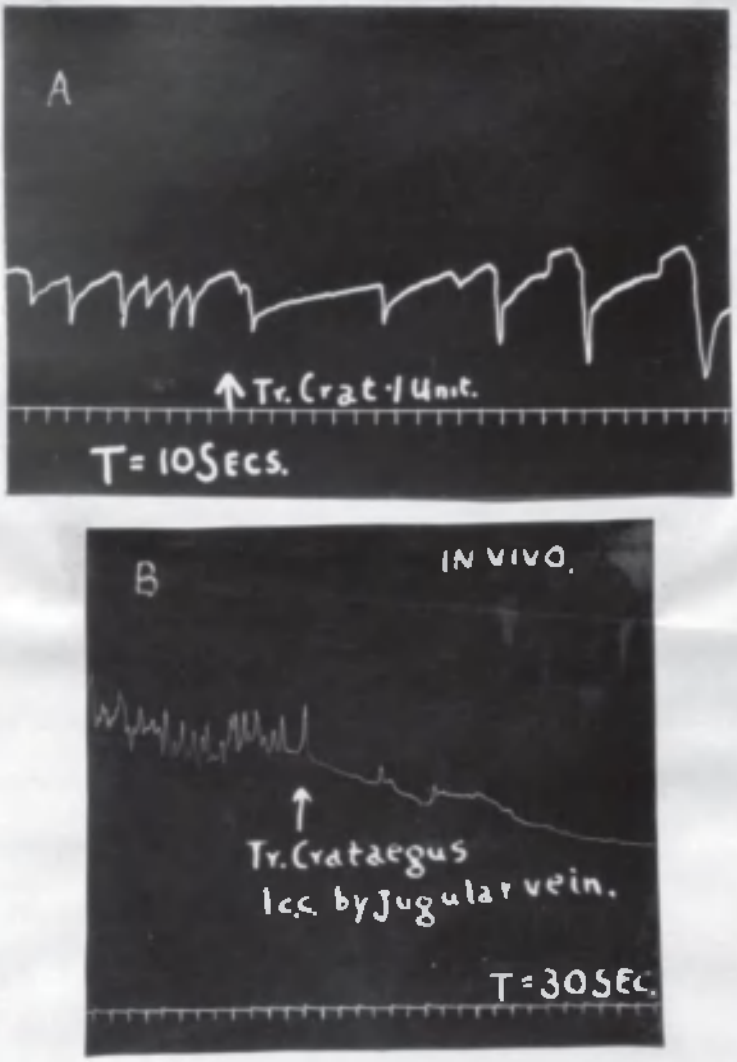

FIG. 26. Effect of Crataegus on Intestine in Vivo.

A. Motor effect on Stomach.

B. Inhibitor effect on Colon.

dissolved and 0.4 cc. injected subcutaneously, i.e. a dose of 0.4 mgm.

11 am. Anaesthesia completed with open ether.
Jugular vein cannulated.
Midline incision made in abdomen, lesser curvature of stomach strung up in special cannula as devised by Bell and described by Bell and Robson (1933), for the uterus. Adjusted to record movements on a slow drum. Abdomen sewen up after installing an electric thermo-couple for regulation of the ttemperature.
12 am. Acetyl choline- 1 cc. of a 1 in 2 millions solution injected intravenously to activate the stomach.
12.15 am. Tincture of Crataegus 0.25 cc. in 1 cc. saline given by the jugular vein. Motor effect marked.

This effect is seen in fig. 26

A similar dose in the intact animal inhibited the tonus and movements of the colon.

According to Liers and Sleeth (1938) and Meyer and Gottlieb (1926) Digitalis increases the gastric motility and cuts down the normal emptying time of the stomach. It would seem as if Crataegus oxyacantha has a similar action.

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THE UTERUS.

1. Uterus in Vitro.

Infusion of Crataegus oxyacantha in dilute concentration (0.5%) has an oxytocic effect on a horn of the pregnant uterus of a guinea pig which has been suspended in warm oxygenated Tyrode-Bayliss solution in a mammalian bowel bath apparatus. In larger doses it is inhibitory.

2. Uterus in Vivo.

Protocol. 23/5/30.

Pregnant female guinea pig, weight 720 gm. Three foeti of the following stature and weight were removed after death.

No.	Length.	Weight.
1.	5.7 cm.	14 gm.
2.	6.0 "	15 "
3.	6.0 "	13 "

10 am. Chloralose 0.5 mgm. subcutaneously.
11 am. " 0.2 " "
12 am. " 0.1 " "

Anaesthesia completed with open ether. Jugular vein cannulated.

Midline abdominal incision made, thermo couple inserted and regulated to 38°C. Left horn of pregnant uterus strung up in special cannula and abdomen closed.

12.30 am. 0.01 unit of pitocin injected intravenously in 1 cc. of saline. Immediate motor effect.

12.45 am. 0.25 cc. Tincture of Crataegus in 1 cc. of Tyrode-Bayliss solution injected intravenously.

FIG. 27. Effect of Crataegus on the Uterus.

A. Inhibitor effect in Vivo.

B. Motor effect in Vitro.

This had an inhibitor effect. Repetition with larger doses has the same effect.

It is thus apparent that the effect of Crataegus oxyacantha on the guinea pig pregnant uterus as recorded in vivo is diametrically opposed to its action in vitro.

This can be seen in fig. 24.

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TOXICOLOGY.

Tincture of Crataegus is a toxic substance, being a cardiac poison. Given by mouth it has little effect on animals in doses which are fatal when given parenterally. The chief seat of its toxicity, and the one which is fatal when large doses are given, or intravenous doses, is the depressant action on the respiratory centre; secondly comes its action on the heart which is more likely to prove fatal in chronic poisoning. There is some evidence for its having a lethal effect through its toxic effects on the liver.

Compared with Standard Tincture of Digitalis it is less powerful when given by mouth, less toxic in its action on the heart, but much more toxic in its effects on respiration. Ignorance of the nature of the specific principle renders its comparison with individual glycosides impossible but it seems to bear a certain relationship to cymarin and antiarin. In some ways it has properties similar to another plant extract - mistletoe. (Henze and Ludivez 1937).

In illustrating these points the following table of comparisons may be of interest:-The extreme toxicity of the intravenous delivery of Crataegus through its action on the respiratory centre which is profoundly depressed, is at once apparent. The relative immunity of frog material is also noticeable.

Table of Lethal Doses.

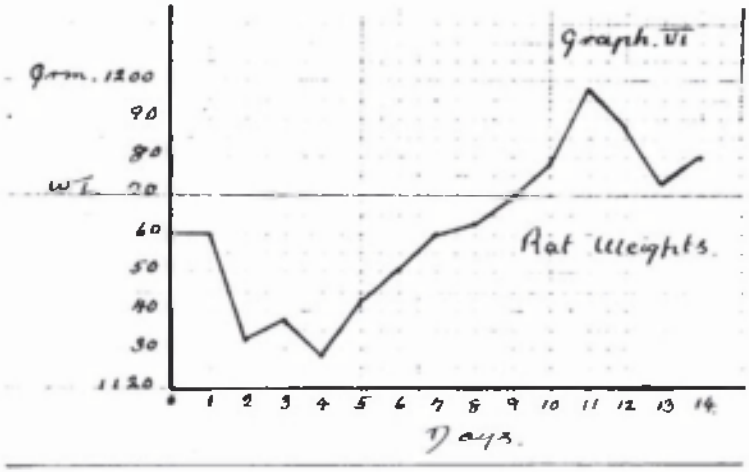
Animal.	Tr. Digitalis. Standard.	Tr. Crataegus. No A.R.	Tr. Crataegus. with A. R.
Frog. R. esculenta.	0.5cc./100gm. (lymph sac)	5.0cc./100gm. (lymph sac)	-
Tortoise.	0.24cc./100gm (i. v.)	2.5cc./100gm. (i. v.)	-
Guinea pig.	0.14cc./100gm. (i. v.)	0.15cc./100gm. (i. v.)	0.34cc./100gm. (i. v.)
Cat.	1.1cc./kilo. (i. v.)	1.0cc./kilo. (i. v.)	3.5cc./ kilo. (i. v.)
Rabbit.	0.56cc./kilo. (i. v.)	1.0cc./kilo. (i. v.)	3.0cc./kilo. (i. v.)
Rat.	0.09cc./100gm. (i. v.)	0.1cc./100gm. (i. v.)	0.47cc./100gm. (i. v.)
Duck.	0.80cc./kilo. (i. v.)	0.30cc./kilo. (i. v.)	3.0cc./kilo. (i. v.)

CHRONIC POISONING.

1. In the Rat.

Five rats of the same brood were taken and a record of their daily weights kept. They were each given 1cc. of tincture of Crataegus in 1cc. of saline subcutaneously each day in order to determine whether or not such treatment would affect the general metabolic state of the animals. The following is a table of their weights:-

Date.	No.1.	No.2.	No.3.	No.4.	No.5.	Total Wts.
27.10.38.	235 gm.	270 gm.	230 gm.	190 gm.	235 gm.	1160 gm.
28.10.38.	234 "	265 "	220 "	188 "	224 "	1131 "
29.10.38.	232 "	259 "	221 "	192 "	235 "	1139 "
31.10.38.	229 "	265 "	218 "	186 "	231 "	1129 "
1.11.38.	233 "	265 "	217 "	206 "	233 "	1144 "
2.11.38.	237. "	270 "	222 "	188 "	233 "	1150 "
3.11.38.	237 "	268 "	221 "	192 "	236 "	1156 "
4.11.38.	237 "	271 "	219 "	193 "	243 "	1163 "
5.11.38.	235 "	275 "	221 "	195 "	241 "	1167 "
7.11.38.	238 "	280 "	224 "	192 "	242 "	1176 "
8.11.38.	247 "	288 "	222 "	192 "	248 "	1197 "
9.11.38.	244 "	281 "	224 "	192 "	247 "	1188 "
10.11.38.	238 "	274 "	222 "	190 "	248 "	1172 "
11.11.38.	240 "	276 "	224 "	196 "	248 "	1184 "


Graph. VI
Gm. 100
90
80
Wt. 70
60
50
40
30
1120
Rat Weights.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days.

It is apparent that the rats at first lost weight heavily due probably to the pain and disturbance of injection, and that they subsequently recovered their position in seven days and went on to gain weight. (Graph VI).

There is thus no generally toxic effect from repeated parenterally administered doses of tincture of Crataegus in rats. There was no evidence of nervous disturbance, loss of balancing power or disorientation such as may be seen in rats poisoned by Digitalis.

At the site of injection of the tincture there developed patches of inflammation with subsequent sloughing of the skin and formation of an indurated sore which only healed very slowly. The animals were accordingly destroyed. Such an effect results from the injection of any tincture and is not specific to Crataegus.

2. In the Guinea pig.

Six guinea pigs, all virgin females, were taken and weighed and put upon a standard diet of bran and greens. Their electrocardiograms were recorded and they were divided into two groups of three. One group was given daily doses of tincture of Crataegus oxyacantha 0.5 cc. per 100 gm. and the other group Diginutin (B.W. & Co) 0.01 cc. per 100 gm. subcutaneously, as follows:-

Group A.			Group B.		
No.	Weight.	Dose.	No.	Weight.	Dose.
1.	480 gm.	2.4cc. Tr. Crataegus.	4.	475 gm.	0.23cc. Diginutin.
2.	380 "	1.9cc. "	5.	420 "	0.21cc. "
3.	325 "	1.6cc. "	6.	405 "	0.20.cc. "

After twelve days necrotic skin lesions began to appear on the back of No. 1. as in the rats. After 14 days the pigs were inactive and off their feed. After 17 days they began to lose hair. By 22 days they had fully recovered their appearance and appetite. After 33 days No. 1. was found dead.

A post mortem examination revealed that the lungs were sound, the usual cause of death in guinea pigs being pneumonia; the heart had stopped in diastole and was fatty; the scars of the necrotic lesions at the sites of injection had healed; the liver had the appearance of fatty degeneration and was dark green in colour; the muscles were stained brown at the injection sites; the gut was thin-walled and yellow in colour.

Injections were carried on with the remaining animals. On the 43 rd. day No. 2. was dead. On the 46 th. day No. 3. was dead.

Post mortem examination revealed similar characters as in No. 1. - fatty liver, heart in diastole, thin yellow bowel, scars at the sites of early injections.

On the 48 th. day the other three, on Diginutin, were killed.

The electrocardiographic records obtained have been examined already (fig. 17).

The liver and heart of each animal was fixed in picro-formal solution for four days, embedded in paraffin wax, cut at 10 u thickness and stained with haemalum and eosin, as was the heart and liver of normal guinea pigs.

According to North and Spung (1937) the cat treated with large doses of Digitoxin develops necrotic lesions in the heart characterised by round cell infiltration and an electrocardiographic response of the "infarct type" or coronary thrombosis type. Loewit (1914) states that cymarin lessens the number of oxydase particles in the guinea pig heart.

Normal Guinea pig Liver.

(a) Low Power.

Glisson's capsule is not well marked. The lobules are confluent. The portal tracts are few and large, the hepatic artery branched, the bile duct double. The hepatic vesicles are obvious.

(b) High Power.

Glisson's capsule consists of a fine endothelium; its interlobular rammifications are very fine. The portal tracts show the usual structures. The liver cells are polygonal with large round nuclei and an obvious intracellular network, particularly just below the capsule. The venous sinuses are large and have scarcely any endothelial wall. The nuclei of the bile duct cells are oval and have a network with four or five nucleoli.

Report on Specimens.

(a) Guinea pig No. 1.

The liver has a more obvious columnar radiating structure of its lobules than the normal specimens. The individual cell columns are obvious and the intercolumnar capillaries distended. The bile duct nuclei are

clear and have lost their network.

(b) Guinea pig No. 2.

Acute yellow atrophy of the liver with massive necrosis of the lobules is seen. This necrosis is collequative and must have existed for at least 24 hours before death. There is some evidence of lymphocytic invasion under the capsule. The nuclei of the bile duct epithelium cells are fragmented.

(c) Guinea pig No. 3.

The same phenomena are seen to a less marked degree in this specimen. There are areas of focal necrosis and central necrosis of the lobules evident throughout with degeneration of bile duct epithelium. There is evidence of hypertrophy of the remaining liver cells.

(d) Guinea pig No. 4.

The lobules of the liver are confluent. The appearance of the liver is within normal limits.

(e) Guinea pig No. 5.

The appearances of the liver are within normal limits.

(f) Guinea pig No. 6.

The appearances of the liver are within normal limits.

Normal Guinea Pig Heart.

The heart of the guinea pig has few distinguishing features.

The epicardium and endocardium are thin and slight. The striated muscle bundles are closely packed and the nuclei pale and large. The bundle of His is not easily distinguished but its large clear pale fibres may be seen

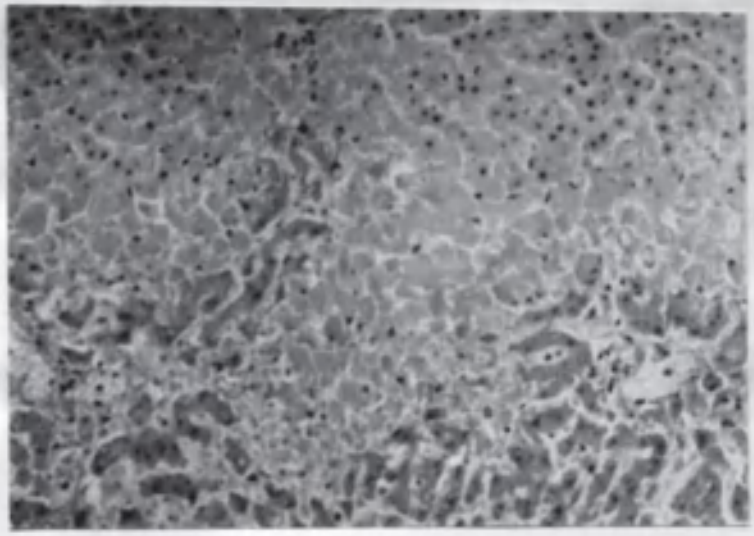


FIG. 28. Effect of Crataegus poisoning on liver of guinea-pig.

Low Power Micro-photograph - necrosis.

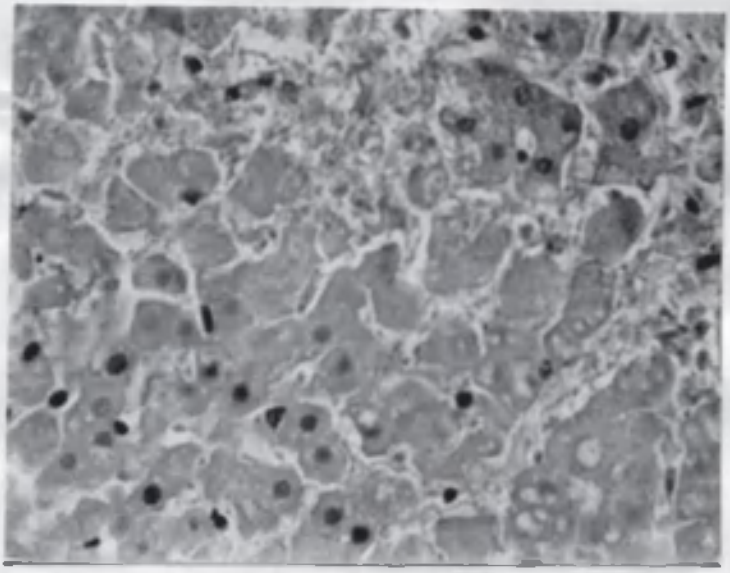


FIG. 29. Effect of Crataegus poisoning on liver of guinea-pig.
High power micro-photograph - necrosis.

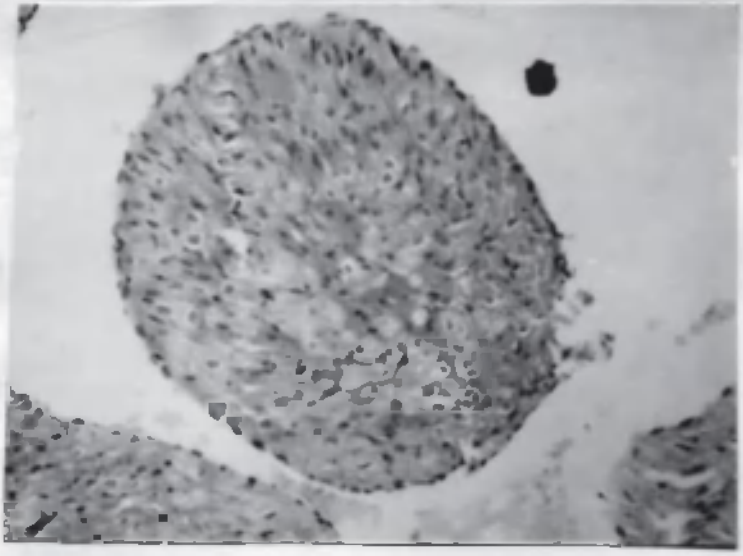


FIG. 30. Effect of Digitalis poisoning on heart of guinea-pig.

Papillary muscle necrosis.

in the interventricular septum. The muscle of the papillary bundles is of the normal cardiac type.

Report on the Specimens.

(a) Guinea pig No. 1.

The appearances of the heart were within normal limits. The bundle of His was very outstanding and appeared to be normal. There was no sign of necrotic lesions.

(b) Guinea pig No. 2.

The appearances of the heart were within normal limits.

(c) Guinea pig No. 3.

The appearances of the heart were within normal limits. There was some indication of fatty infiltration of the right ventricular wall.

(d) Guinea pig No. 4.

The appearances of the heart were within normal limits.

(e) Guinea pig. No. 5.

The appearances of the heart were within normal limits.

(f) Guinea pig No. 6.

The papillary muscle indicated necrotic change with a degree of fibrosis.

Chronic poisoning of the guinea pig with tincture of Crataegus oxyacantha leads to death from liver necrosis. There are no apparent morphological changes in the heart muscle. Digitalis has no visible effect on the liver cells but may cause some degree of fibrosis in the heart.

These changes are illustrated in Fig.28,29,30

METHODS OF ASSAY.

Before undertaking any therapeutical investigations with a drug so toxic as Crataegus oxyacantha, some estimate of the potency relative to known preparations was essential. Accordingly it was determined to prepare a large volume of tincture of Crataegus by the maceration process as previously described, and assay it biologically against standard tincture of Digitalis.

There are now a considerable number of methods of biological assay of Digitalis, and also of chemical methods. It was decided to carry out the chemical method of Knudson and Dresbach (1923) and the biological methods using the frog and the cat.

Of these, the chemical method, while of interest is not of great significance since the chemical nature of the active principle of Crataegus is not known and indeed must be of quite different nature to the "digitaloid" glycosides, from such information as has been gained on the subject.

1. CHEMICAL METHOD.

The method of Knudson and Dresbach (1923) is the logical outcome of several previous methods which met with little success. Of these, that of Martindale (1912) consisted of a colourimetric reaction of the active constituents of the preparation under investigation with Froehde's reagent - sulphuric ammonium molybdate. The method took three hours to carry out and only estimated the approximate activity of the preparation.

The method of Beery (1919) was based on the previous method, with differences in the method of extraction of the preparation and working up of the solution.

There are in this method two colourimetric processes A and B. A estimates the water soluble glycosides while B estimates the total glycosides. In process A digitoxin and saponin are eliminated and the colourimetric estimation gives the therapeutic value. B - A gives the toxic value of the tincture. It has not been shown by either of these investigators that the colour reaction varies in accordance with the physiological activity of the glycosides.

Baljet (1918) evolved a method of colourimetric estimation of the "digitaloid" glycosides with picric acid and sodium hydroxide. Baljet believes that the lactone function in the molecule of the glycoside is essential in producing the test reaction. Baljet observed that the glycosides react with increasing intensity of colour in the order of their physiological activity.

The method of Knudson and Dresbach is as follows:-

Solutions.

1. 10% solution of neutral lead acetate.

2.5 cc. to each determination.

2. 10% solution of di-sodium hydrogen phosphate.

1.25 cc. to each determination.

3. Alkaline picrate solution.

95 cc. of 1% purified picric acid solution with 5 cc. of

10% sodium hydroxide.

4. The Standard for comparison.

This is 0.266 mgm. of crystalline ouabain in 5 cc. of water. 5 cc. of this solution with 5 cc. of alkaline picrate give a colour equivalent to 0.5 cat unit.

Method.

5 cc. tincture of Crataegus oxyacantha in a 25cc. flask diluted to 15 cc. with distilled water. 2.5 cc. lead acetate solution (1) added, mixed, diluted with water to the mark, shaken and filtered.
12.5 cc. of filtrate are put in a flask with 1.25 cc. of di-sodium hydrogen phosphate solution (2), diluted to the 25 cc. mark, mixed, and filtered. The filtrate is clear. 5 cc. of this filtrate is put in a 10 cc. volumetric flask and 5 cc. of the solution of the Standard (4) put in another 10 cc. volumetric flask. To each is added 5 cc. of alkaline picrate solution (3) mixed, stood for 25 minutes, and then compared in a colourimeter at 20 mm. depth.

Since the calculation demands a knowledge of the number of milligrammes of the pure drug in the unknown solution, and this was unknown, a change in the method had to be made.

Accordingly the ouabain Standard was compared with tincture of Crataegus and with 1926 Standard Tincture of Digitalis prepared as follows:-

One ampoule (½ gm.) of International Digitalis Standard (1936) powder having been received from the

Department of Biological Standards, the National Institute for Medical Research, Hampstead, London NW3, was stored at 4°C for 7 days. The ampoule was opened and its contents weighed in a dry stoppered bottle: weight of powder = 3.00 gm. The powder was placed in the capsule of a Soxhlet reflux condenser and extracted with 80 cc. of absolute methyl alcohol for 6 hours. This was evaporated to a volume of 15 cc. and made up to 30 cc. with glass distilled water, giving 30 cc. of Standard (1936) Tincture of Digitalis which is equivalent to 37.5 cc. of the original Standard (1926) Tincture of Digitalis. The appropriate dilution having been made the 1926 preparation was used as the Standard Preparation for purposes of assay by the chemical method of Knudson and Dresbach, the frog method and the cat method of Hatcher and Brody.

This Standard Tincture having been prepared according to the instructions given was used as the unknown in a series of estimations by the method of Knudson and Dresbach against the ouabain Standard. Tincture of Crataegus was similarly treated. The figures given by the two tinctures were then compared, giving a measure of their relative potencies. The comparison was carried out at 20 mm. depth in a Pulfrich photometer using the No. 9. yellow filter.

Results.

1. Standard at 20 mm.

Tincture of Crataegus matches at 25 mm.

2. Standard at 20 mm.

20% Standard Tincture of Digitalis matches at 17.5 mm.

25/20 = 87.5/20

Tr. Crataegus/Tr. Digitalis = 25/87.5 = 1/3.5

Tincture of Crataegus has a potency of 28.57% of International Standard (1926) Tincture of Digitalis.

2. THE FROG METHOD.

In the frog method of assay an injection is made under the skin of the frog and it is left for 1 hr., 3 hrs., or overnight, according to the period preferred. While the 1 hr.,method is official in the United States Pharmacopoeia many workers agree that the 12 hr. or overnight period enables a sharper distinction to be drawn between the dead frogs and the survivors.

The Standard Tincture of Digitalis was first treated as follows:- 10 cc. were put on a water bath and the volume reduced to 5 cc. at a temperature of 50°C. and restored to 10 cc. with 0.6% NaCl solution. The tincture of Crataegus was similarly treated.

The frogs must all be of the same sex for one part of the test, though either sex may be used except females preparing for spawning. The results are more uniform if frogs in winter time are used. The frogs must be weighed and kept in pairs, one of a pair receiving the Standard and the other the unknown, in a dose according to the body weight. Fromherz (1936) has shown that when doses are adjusted according to body weight heavier frogs

appear to be more sensitive than lighter ones. This factor is usually ignored. According to Trevan (1927) discrepant results in biological assay must not be discarded except on the clearest evidence of an error in technique.

The drugs having been prepared in suitable dilutions relative to the weights of thefrogs which are kept in glass jars labelled with the weight to within 1 gm., the injection is made by holding the animal on its back by the left hand with a cloth and stretching a leg with the finger and thumb. The right hand can then pass a needle through the muscles of the thigh and bring it up under the skin of the abdomen and make the injection.

Protocol. Assay No. 1.

24.1.38. Twenty pairs of male frogs were brought in from the outside storage tank to the laboratory and left to adjust themselves to the heightened temperature.

25.1.38. The frogs were weighed and arranged in pairs under glass bell jars on moist blotting paper at an equable temperature. The drugs were prepared and injections made as follows:-

Table of preliminary test of assay of Tincture of Crataegus.

Pair No.	Weight.	Dosage of Drugs injected. Tr. Digitalis. 0.006cc./gm.	Tr. Crataegus. 0.012cc./gm.	Time.	12 hr. result. Dig.	Crat.
1.	31.0 gm.	0.186 cc.	0.37 cc.	11.30 a.m.	Dead	Alive
2.	21.7 "	0.14 "	0.28 "	11.35 "	"	"
3.	18.5 "	0.11 "	0.22 "	11.40 "	"	"
4.	24.0 "	0.145 "	0.29 "	11.45 "	"	"
5.	25.0 "	0.15 "	0.30 "	11.50 "	Alive	"
6.	22.0 "	0.132 "	0.26 "	11.55 "	Dead	"
7.	26.5 "	0.16 "	0.32 "	12.0 "	"	"
8.	17.0 "	0.10 "	0.20 "	12.5 "	"	"
9.	18.5 "	0.11 "	0.22 "	12.10 "	"	"
10.	28.0 "	0.168 "	0.336 "	12.15 "	"	"
11.	21.0 "	0.126 "	0.252 "	12.20 "	"	"
12.	21.8 "	0.13 "	0.26 "	12.25 "	"	"
13.	19.7 "	0.118 "	0.236 "	12.30 "	"	"
14.	33.0 "	0.132 "	0.264 "	12.35 "	"	"
15.	21.0 "	0.126 "	0.252 "	12.40 "	"	"
16.	25.0 "	0.15 "	0.30 "	12.45 "	"	"
17.	21.6 "	0.123 "	0.246 "	12.50 "	"	"
18.	17.0 "	0.10 "	0.20 "	12.55 "	"	"
19.	18.5 "	0.11 "	0.22 "	1.0 p.m.	"	"
20.	25.0 "	0.15 "	0.30 "	1.5 "	"	"

It is apparent from this preliminary test that 0.006 cc. Standard Tincture of Digitalis per gramme of frog (Rana temporaria) gave a mortality of 95% while 0.012 cc. Tincture of Crataegus oxyacantha per gramme frog had a mortality of 0%.

Further tests with "sighting doses" were thus required.

Protocol. 23.1.38.

Four male frogs were chosen for sighting doses.

Pair No.	Weight.	Dose.	Result.
1.	27.3 gm.	0.65 cc.	Alive.
2.	18.7 "	0.45 "	"
3.	29.2 "	0.70 "	"
4.	17.4 "	0.42 "	"

0.024 cc. of tincture of Crataegus/ gm. frog is not enough.

This was repeated with larger doses as follows:-

Pair No.	Weight.	Dose.	Result.
1.	25.0 gm.	1.0 cc.	1 Alive.
2.	25.0 "	1.0 "	2 Dead.
3.	27.7 "	2.2 "	2 "
4.	34.1 "	2.7 "	2 Alive
5.	28.2 "	2.25 "	2 Dead.
6.	25.9 "	2.05 "	2 "

0.04 cc. of tincture of Crataegus / gm. frog = 25% mortality

0.07 " " " " " " " = 75% "

Protocol. 24.1.38.

Pair No.	Weight.	Dose.	Results.
1.	20.6 gm.	1.65 cc.	2 Dead.
2.	17.2 "	1.37 "	2 "
3.	27.0 "	2.16 "	2 Alive.
4.	11.5 "	0.56 "	2 Dead.

0.08 cc. of tincture of Crataegus / gm.frog = 75% mortality

Making use of the information gathered from these attempts to find the 50L.D. for frogs the assay was repeated with the following results:-

Protocol. 25.1.38.

		Dosage.		12 hr. Result.	
Pair No.	Weight.	Tr. Digitalis 0.005cc./gm.	Tr. Crataegus 0.05cc./gm.	Time.	Dig. Crat.
1.	26.2 gm.	0.131 cc.	1.31 cc.	10.00 am.	Dead. Dead.
2.	19.7 "	0.0985 "	0.985 "	10.3 "	" Alive
3.	18.5 "	0.0925 "	0.925 "	10.6 "	" Dead
4.	17.7 "	0.0885 "	0.885 "	10.9 "	Alive Alive
5.	23.0 "	0.115 "	1.15 "	10.12 "	Dead Dead
6.	16.7 "	0.0835 "	0.835 "	10.15 "	Alive "
7.	19.3 "	0.0985 "	0.985 "	10.18 "	" "
8.	18.5 "	0.0925 "	0.925 "	10.21 "	" "
9.	21.0 "	0.105 "	1.05 "	10.24 "	Dead Alive
10.	31.0 "	0.155 "	1.55 "	10.27 "	Alive "
11.	18.6 "	0.093 "	0.93 "	10.30 "	Dead Dead
12.	21.7 "	0.1085 "	1.085 "	10.33 "	" Alive
13.	21.7 "	0.1085 "	1.085 "	10.36 "	" Dead
14.	26.7 "	0.1335 "	1.335 "	10.39 "	Alive Alive
15.	26.7 "	0.1335 "	1.335 "	10.41 "	Dead "
16.	21.0 "	0.105 "	1.05 "	10.44 "	" "
17.	21.0 "	0.105 "	1.05 "	10.47 "	" Dead
18.	22.5 "	0.1125 "	1.125 "	10.50 "	Alive Alive
19.	27.0 "	0.135 "	1.35 "	10.53 "	." Dead
20.	22.5 "	0.1125 "	1.125 "	10.56 "	" Alive

50 L. D. is represented as having a potency of 100.

Thus 0.05 cc. Tincture of Crataegus = 100/100 times 0.005 cc. tincture of Digitalis

or Tr. Crataegus = 10% Standard Tincture of Digitalis.

The following winter this work was repeated in so far as it applied to the tincture of Crataegus oxyacantha in order to determine whether the tincture had kept its potency during that year of storage in bulk in Winchester bottles at 4°C.

Accepting that the 50 L. D. for Rana temporaria in this laboratory was 0.005 cc. Standard Tincture of Digitalis per gramme frog the work on Crataegus was repeated in some detail. As Rana temporaria was not available Rana esculenta was used. Edmunds, Moyer and Shaw (1937) declare:- "It would seem therefore that regardless of the period of observation (one hour or twelve hours) or the variety of frogs (r. pipiens or r. temporaria) the characteristic curve for digitalis has the same slope and that the two species of frogs display the same degree of variation to toxic doses of digitalis(for the lymph sac method of injection). These findings would therefore seem to justify the use of the statistical method when either species of frog is used or either period of observation without first establishing an integrated curve in the individual laboratory".

Protocol. 21.11.38.

Sixty male frogs(R. esculenta) were brought into the laboratory for twelve hours to adjust themselves to the higher temperature.

22.11.38. They were weighed, injected with 0.05 cc. tincture of Crataegus per gramme frog and observed after twelve hours with the following results.-

No.	Weight.	Dose 0.05cc./gm.	12 hrs.result.	Time.
1.	26 gm.	1.3 cc.	Alive.	2.0 pm.
2.	32 "	1.6 "	Dead	2.2 "
3.	32 "	1.6 "	Alive	2.4 "
4.	42 "	2.1 "	Dead	2.6 "
5.	24 "	1.2 "	"	2.8 "
6.	30 "	1.5 "	Alive	2.10 "
7.	30 "	1.5 "	"	2.12 "
8.	30 "	1.5 "	Dead	2.14 "
9.	24 "	1.2 "	"	2.16 "
10.	28 "	1.4 "	"	2.18 "
11.	28 "	1.4 "	Alive	2.20 "
12.	30 "	1.5 "	Dead	2.22 "
13.	20 "	1.0 "	Alive	2.24 "
14.	24 "	1.2 "	Dead	2.26 "
15.	26 "	1.3 "	Alive	2.28 "
16.	26 "	1.3 "	Dead	2.30 "
17.	28 "	1.4 "	"	2.32 "
18.	22 "	1.1 "	"	2.34 "
19.	24 "	1.2 "	Alive	2.36 "
20.	22 "	1.1 "	"	2.38 "
21.	25 "	1.25 "	Dead	2.40 "
22.	26 "	1.3 "	"	2.42 "
23.	30 "	1.5 "	"	2.44 "
24.	26 "	1.3 "	Alive	2.46 "
25.	26 "	1.3 "	Dead	2.48 "
26.	28 "	1.4 "	"	2.50 "
27.	22 "	1.1 "	"	2.52 "

No.	Weight.	Dose 0.05cc./gm.	12 hrs.result.	Time.
27.	22 gm.	1.1 cc.	Alive.	2.54 pm.
28.	30 "	1.5 "	Dead.	2.56 "
29.	24 "	1.2 "	"	2.58 "
30.	26 "	1.3 "	"	3.0 "
31.	26 "	1.3 "	Alive.	3.2 "
32.	32 "	1.6 "	Dead.	3.4 "
33.	26 "	1.3 "	"	3.6 "
34.	26 "	1.3 "	"	3.8 "
35.	32 "	1.6 "	Alive.	3.10 "
36.	24 "	1.2 "	"	3.12 "
37.	40 "	2.0 "	Dead.	3.14 "
38.	30 "	1.5 "	"	3.16 "
39	20 "	1.0 "	"	3.18 "
40.	30 "	1.5 "	"	3.20 "
41.	46 "	2.3 "	"	3.22 "
42.	24 "	1.2 "	"	3.24 "
43.	34 "	1.7 "	"	3.26 "
44.	25 "	1.25 "	"	3.28 "
45.	26 "	1.3 "	"	3.30 "
46.	32 "	1.6 "	"	3.32 "
47.	30 "	1.5 "	Alive.	3.34 "
48.	24 "	1.2 "	Dead.	3.36 "
49.	20 "	1.0 "	"	3.38 "
50.	26 "	1.3 "	Alive.	3.40 "
51.	28 "	1.4 "	Dead.	3.42 "
52.	28 "	1.4 "	"	3.44 "
53.	24 "	1.4 "	Alive.	3.46 "

No.	Weight.	Dose 0.05cc./gm.	12 hrs.result.	Time.
54.	24 gm.	1.2 cc.	Dead.	3.48 pm.
55.	24 "	1.2 "	Alive.	3.50 "
56.	26 "	1.3 "	Dead.	3.52 "
57.	18 "	0.9 "	"	3.54 "
58.	24 "	1.2 "	"	3.56 "
59.	26 "	1.3 "	"	3.58 "
60.	30 "	1.5 "	"	4.0 "
Total.	1626 gm.	81.7 cc.	79% mortality.	2 hrs.

79% mortality according to Trevan's characteristic curve gives a potency of 114.

Now if 0.005 cc. Standard Tincture of Digitalis = 50 L.D. = 100 potency,

0.05 cc. tincture of Crataegus = 114/100 times 0.005 cc. Standard, or

1 cc.tincture of Crataegus = 0.114 cc. Standard, or Tincture of Crataegus oxyacantha = 11.4% potency of Standard Tincture of Digitalis.

Although such a result id within the limits of experimental error it may be safer to assume that there are differences in the resistance to toxicity of R. temporaria and R. esculenta.

Using the characteristic curve worked out by Chapman and Morrell (1931) the results are even closer, giving a percentage of 10.55% of the Standard.

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3. The Cat Method.

The cat method of Hatcher and Prody (1910) was modified by de Lind van Wijngaarden (1926) and subsequent to this there has been much discussion of details of the technique, which has been previously reviewed.

The method adopted was as follows.

A cat is weighed: it must not be pregnant or lactating and the weight must be between 1.7 and 3.0 kilos. It is given 0.5 grains of nembutal per kilo of cat subcutaneously and after one hour a little ether while tracheotomy is performed, the jugular vein cannulated and a cannula inserted into the carotid artery in order to record the blood pressure. After some time artificial respiration is begun and at the same time the continuous intravenous infusion of Standard (1926) Tincture of Digitalis diluted twenty times with Tyrode-Bayliss solution warmed to 38° C by circulation round a heating coil of glass. The infusion comes from a burette which is converted into a Marriotte flask by fitting a cork and a capillary tube which stretches below the graduations. A record of the time of infusion and of the blood pressure is taken on the kymograph. Matters are so arranged that the infusion takes 30-45 minutes to cause death. The lethal dose is noted, a post mortem examination done, and the heart excised, cleaned and weighed. Bond (1927) states that if the results of a cat assay are based on the heart weight (free from clot) rather than the total weight of the cat there is 50% less error due to individual

deviation as the heart weight is related to the total weight in a constant relation whereas the total weight may contain a large dead weight of fatty tissue.

There are various opinions as to the number of cats which must be used in order to obtain significant results; it was decided to find the lethal dose of the Standard on four cats and the unknown on six cats and to compare the potencies as assayed by the L.D. in cc. per kilo of cat and the L.D. in cc. per gramme of heart weight. The results were as follows:-

As cats do not vary in their reaction to toxic doses of digitalis with the season the dates of performance of the tests were immaterial but all were performed within fourteen days.

Standard (1926) Tincture of Digitalis.

Cat No.	Weight.	Time.	Heart Wt.	L.D./kilo.	L.D./gm. Heart
1.	3.0 kilo.	35 mins.	16.6 gm.	1.038 cc.	0.1876 cc.
2.	1.8 "	40 "	9.0 "	1.470 "	0.2933 "
3.	3.0 "	36 "	12.85"	1.379 "	0.3304 "
4.	2.5 "	36 "	13.8 "	1.400 "	0.2536 "

Tincture of Crataegus.

Cat No.	Weight.	Time.	Heart Wt.	L.D./kilo.	L.D./gm. Heart
1.	2.8 k.	35 mins.	12.0 gm.	6.60 cc.	1.540 cc.
2.	3.0 "	38 "	15.0 "	6.75 "	1.150 "
3.	2.1 "	38 "	9.5 "	6.60 "	1.459 "
4.	1.8 "	40 "	8.7 "	7.65 "	1.590 "
5.	1.6 "	35 "	9.0 "	9.39 "	1.669 "
6.	2.3 "	38 "	12.7 "	6.50 "	1.177 "

Mathematical Treatment of Results.

L.D. of Standard Tincture for 4 cats/kilo.

= 1.038 cc, 1.470 cc., 1.379 cc, 1.48

Mean = 1.32175 cc. per kilo of cat.

Standard Error = $\sqrt{\Sigma d^2/n(n-1)}$ = $\sqrt{0.1191/12}$ = $\sqrt{0.009926}$

= 0.09657 cc.

L.D. of Standard = 1.32175 ± 0.09657 cc./ kilo.

Standard Error = 7.30% of L.D.

L.D. of Standard Tincture for 4 cats per gm. heart

= 0.1876 cc, 0.2933 cc., 0.3304 cc., 0.2536 cc.

Mean = 0.2662 cc./ gm. Heart weight.

Standard Error = $\sqrt{\Sigma d^2/n(n-1)}$ = $\sqrt{0.0119/12}$ = $\sqrt{0.0009925}$

= 0.0354 cc. per gramme.

L.D. Standard = 0.2662 ± 0.0354 cc./gm. Heart weight.

Standard Error = 11.47% of L.D.

L.D. Tincture of Crataegus / kilo of cat, for 6 cats

= 6.6 cc., 6.75 cc., 6.6 cc., 7.65, 9.39, 6.5

Mean = 7.2483 cc. per kilo of cat.

Standard Error = $\sqrt{\Sigma d^2/n(n-1)}$ = $\sqrt{6.405/30}$ = $\sqrt{0.215}$

= 0.4620 cc. per kilo of cat.

L.D. Tincture of Crataegus = 7.2483 ± 0.4620 cc./kilo.

Standard Error = 6.37% of L.D.

L.D. Tincture of Crataegus per gm. heart for 6 cats.

= 1.54 cc., 1.15 cc., 1.459 cc., 1.59 cc., 1.6693

1.117 cc.

Mean = 1.4309 cc. per gramme heart weight.

Standard Error = $\sqrt{\Sigma d^2/n(n-1)}$ = 0.1916/30 = 0.006386

= 0.07991 cc. / gm.

L.D. Tincture of Crataegus = 1.4309 ± 0.07991 cc./ gm.

Standard Error = 5.64% of L.D.

From these assays it is obvious that:-

1. According to the method of Hatcher and Brody Tincture of Crataegus oxyacantha = 18.2% potency of Standard (1926) Tincture of Digitalis.

2. According to the method of Bond Tincture of Crataegus oxyacantha = 18.88% potency of Standard (1926) Tincture of Digitalis.

Examination of the ratios of the standard errors of these results to the assayed lethal doses is in favour of the original Hatcher method rather than Bond's modification, although the ultimate difference is but little.

Collection and comparison of the different methods of assay gives the following results:-

Potency of Tincture of Crataegus in terms of Digitalis:-

Frog	1.	11.40%
"	2.	10.0 %
Cat	1.	18.2%
"	2.	18.88%
Chemical		28.57%
Mean		17.41%

Since the chemical composition of the essential principles of the two tinctures is indubitably different the chemical assay may be ignored, and we get a Mean Potency = 14.63%.

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THERAPEUTICS.

Pharmacological investigations having shown that Crataegus oxyacantha contained ans active principle of the nature of Digitalis and having indicated some of its properties it remained to try its therapeutic effects in the clinique. As was previously stated the majority of the literature dealing with Crataegus oxyacantha consists of a series of more or less detailed reports of the action of tincture of Crataegus or of fresh infusion of Crataegus as a nerve sedative, a cardiac tonic, a potent agent in lowering the blood pressure and as a cure for sundry respiratory conditions. These papers contain no precise figures and but seldom are any actual case histories mentioned. For the most part they are more or less verbatim reports of short speeches made or questions asked at medical conferences. It was thus obvious that in approaching patients in the wards extreme care had to be taken. It was known that in large doses given intravenously the tincture was lethal as a result of its toxic action on the heart and respiratory centre and that given parenterally it would affect the liver after some time. Of its administration by mouth little was known except that it tended to be anti-diuretic to the rat. The vaso dilation and profound fall in blood pressure which accompanied intravenous administration were said to be found also on administration by mouth, according to various writers but this had not been confirmed experimentally. The chief and obvious safe-

guard was therefore the guide as to dosage afforded by the results of biological assay. According to assay tincture of Crataegus oxyacantha had a potency of 14.6% of Standard 1926 Tincture of Digitalis.

According to the British Pharmacopoeia (1934) the dosage of tincture of Digitalis is as follows:-

Single dose 0.5 -2.0 drams.

Repeated " 5.0 - 15.0 minims.t. d. s.

Thus the safe dosage of tincture of Crataegus might be taken as Single dose 3.0 - 12.0 drams.

Repeated " 0.5 - 1.5 " .

Therapeutic Trials.

(a) Auricular Fibrillation.

Digitalis finds its chief sphere of usefulness in cases with tachycardia and irregularity of the heart's action, with or without cardiac decompensation and oedema. It might therefore be presumed that Crataegus oxyacantha would have some effect on cases suffering from auricular fibrillation, and that this action would resemble that of Digitalis. The following cases of auricular fibrillation were investigated.

Case 1. J. L., male, aet. 52 yrs. Admitted 12.5.34.

Complaint. Breathlessness with swelling of the ankles since May 1934.

History. As a child the patient had Scarlet fever, in 1918 severe influenza. He has had no rheumatism or sore throats. Since then he has suffered from oedema, breathlessness and dizzy turns, cough and frothy sputum,

beginning in 1934. He has been in hospital several times. The urinary volume is very variable.

His father died of angina pectoris.

Examination. 12.5.38.

Well nourished man, slight cyanosis, oedema of feet and ankles.

Alimentary System. Liver 1.5 inches below the costal margin and tender to the touch; otherwise N.A.D.

Cardio-vascular System. Pulse regular, soft, rate 120 per minute. Vessels not palpable. B.Pr. 180/95 mm. Hg. Apex beat not visible or palpable. Sounds pure but distant. Apex in 6 th. interspace 4.75 inches from mid-line.

Respiratory System. Dulness at right base.

Central Nervous System. N.A.D.

Urine. Sp. Gr. 1020. Albumin 0.25 parts Esbach. No casts

Blood. R.b.c. 6.0 mils. Hb. 100%. Blood Urea 90 mgm.%

W.b.c. 9,400 C.I. 0.9 W.R. - ve.

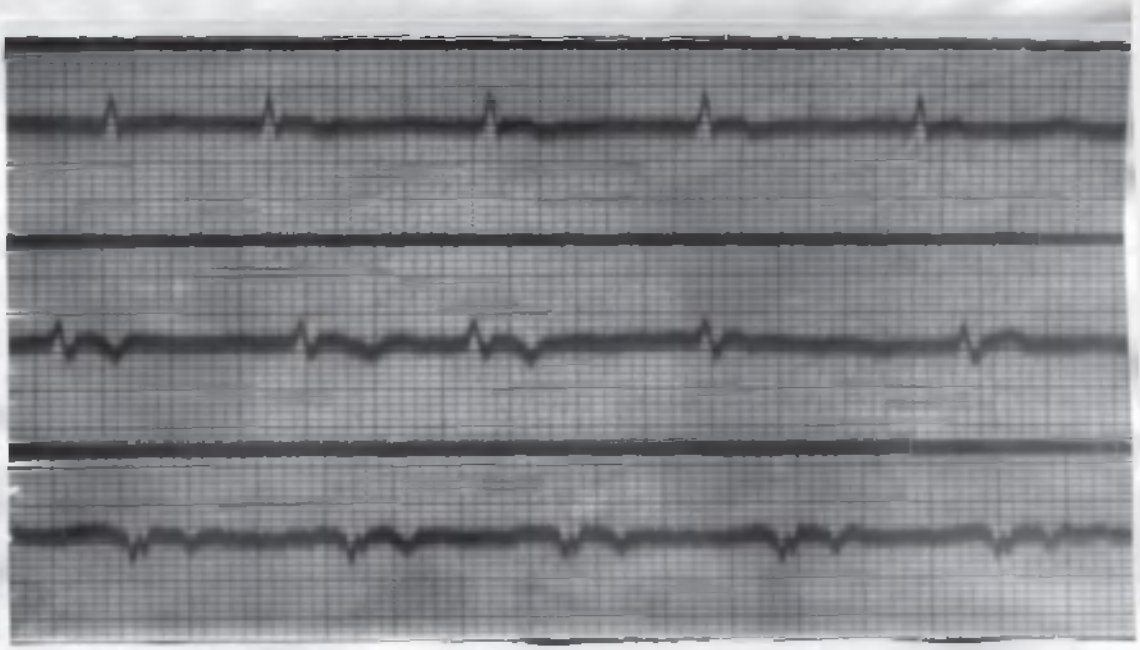
Case Record.

16.5.38. Fluid in right chest to lower angle of scapula. Fulv. Digitalis gr.4 per day.

18.5.38. Paracentesis of chest. 20 cc. of straw-coloured fluid removed. Digitalis stopped due to sickness. Urea clearance 60%. Van den Bergh test biphasic direct positive. Indirect 8 units. X-ray right pleural effusion. Heart enlarged to left and right sides.

21.5.38. Oedema and albumin increased. Cardiac sounds pure.

FIG. 31. Electrocardiogram of J.L.
Auricular fibrillation.



23.5.38. Pulv. Dig. gr.1 t.d.s. Salyrgan 1 cc. every second day intramuscularly.

27.6.38. Digitalisation interrupted by sickness several times. Dyspnoea severe.

12.8.38. Southey's tubes inserted in legs. 13 pints of fluid removed. Put on Tab. Nativelle gr. 1/400 b.d.

24.10.38. Treatment with Tincture of Crataegus begun.

25.10.38. Blood urea 40 mgm.%.

Dyspnoea, cyanosis, jaundiced appearance. Laryngeal catarrh. Superficial sepsis of the right hand. Oedema of feet and legs to the knees and of sacral pad.

Cardio-vascular System. Pulse of poor quality, low tension, irregular, intermitting, and with palpable vessels. Electrocardiogram - auricular fibrillation. (see Fig. 31).

B.Pr. 145/84.

Apex - right border 12.0 cm. Liver in nipple line 14 cm. Liver tender on palpation. Apex - mid line 10.0 cm . Sounds distant and pure. Second aortic sound slapping. Apex rate 100 per minute. Radial pulse 72 per minute.

Other Systems. Right base dull. Ascites in abdomen. Umbilicus - pubis 16.0 cm.

26.10.38. Apex rate 130 per minute. Radial pulse 104 per minute. Less cyanosis and icterus: slept well. Cheyne-Stokes respiration in sleep. Mucus and blood in stools.

27.10.38. Icterus gone. Cyanosis less. Apex - right border 12.0 cm. Liver 15.0 cm., not so tender. Apex rate 102 per minute. Radial pulse 84 per minute.

B.Pr. 128/70. mm. Hg.

28.10.38. Apex rate 100 per minute. Radial pulse 92 per minute. Oedema unchanged. Umbilicus - pubis 15 cm. Liver 13.0 cm. and no longer tender. Appetite good.

31.10.38. Icterus gone. Still orthopnoeic. B.Pr. 116/75. Apex rate 100 per minute. Radial pulse 100 per minute. Beats stronger, less intermissions, sounds pure.

1.11.38. Oedema of legs gone. Vomited dose at 6 p.m. on 31.10.38. Not so well. Apex rate 108 per minute. Radial pulse 108 per minute.

2.11.38. Apex rate 108 per minute. Radial pulse 108 per minute. Dulness of liver 1 cm. less. Umbilicus - pubis 14.0 cm. Oedema of legs gone. Treatment stopped.

5.11.38. Tab. Dig. Folium. gr. 1. 4.in.die. Cough troublesome. Liver 16 cm. Apex - right border 11 cm. Umbilicus - pubis 13.0 cm.

7.11.38. B.PR. 145/95. mm. Hg. Pleural rub at right lung base: fluid gone.

9.11.38. B.Pr. 140/85. mm. Hg.

10.11.38. Electro cardiogram unchanged.

11.11.38. B.Pr. 142/90. mm. Hg. Umbilicus -pubis 13 cm. No oedema of legs. No fluid in chest.

Water Balance.

Date.	In.	Out.	Date.	In.	Out.
23.10.38.	62.	39 oz.	1.11.38.	79	62 oz.
24.10.38.	60.	44 "	2.11.38.	72.	52. "
25.10.38.	48.	56. "	3.11.38.	72.	64. "
26.10.38.	48.3	38. "	4.11.38.	90.	79. "
27.10.38.	46.	46. "	5.11.38.	80.	68 "

Date.	In.	Out.	Date.	In.	Out.
28.11.38.	70	66 oz.	6.11.38.	88.	98. oz.
29.11.38.	78.	43 "	7.11.38.	80.	78. "
30.11.38.	78.	60. "	8.11.38.	90.	100 "
31.10.38.	65.	40."	9.11.38.	84.	60. "

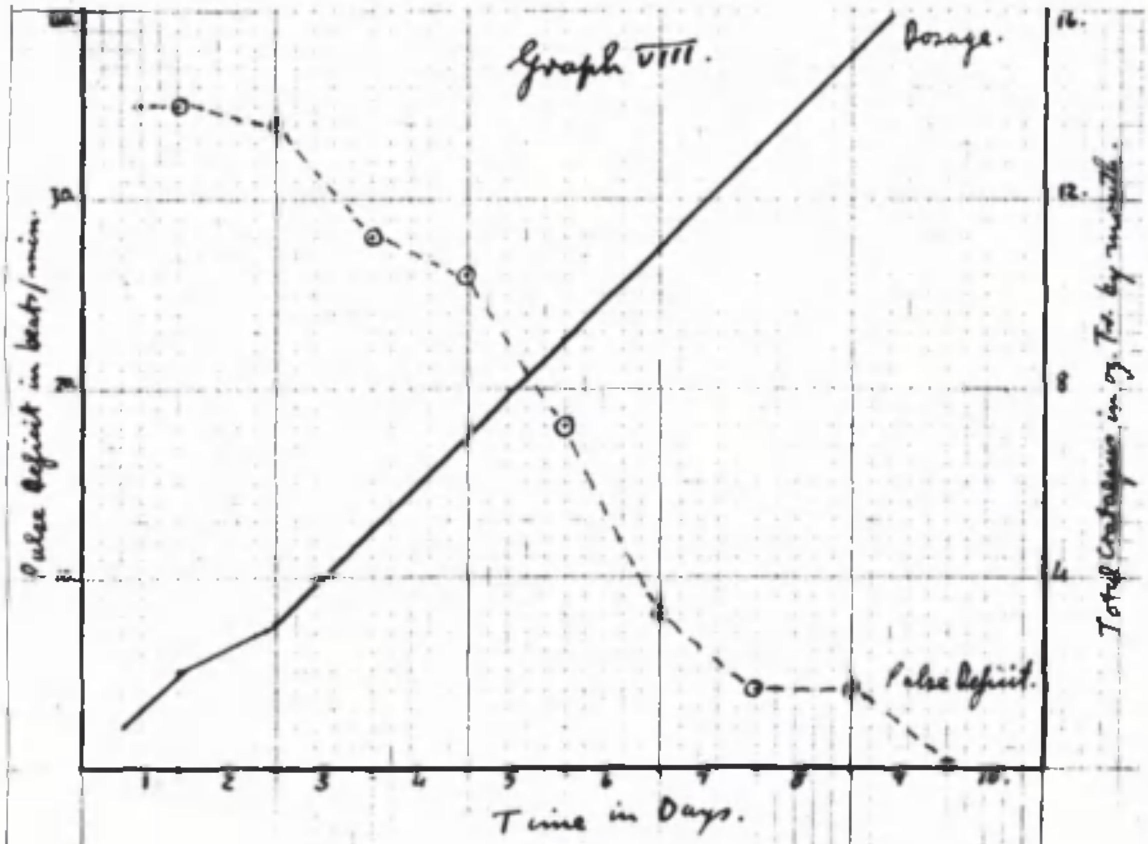
Treatment.

24.10.38.	Tr. Crataegus	0.5	oz.	hourly.	for	4	doses.	-	2.0
25.10.38.	"	"	0.5	"	6	"	" 2	"	- 1.0
26.10.38.	"	"	0.5	"	6	"	" 4	"	- 2.0
27.10.38.	"	"	0.5	"	6	"	" 4	"	- 2.0
28.10.38.	"	"	0.5	"	6	"	" 4	"	- 2.0
29.10.38.	"	"	0.5	"	6	"	" 4	"	- 2.0
30.10.38.	"	"	0.5	"	6	"	" 4	"	- 2.0
31.10.38.	"	"	0.5	"	6	"	" 4	"	- 2.0
1.11.38.	"	"	0.5	"	6	"	" 4	"	- 2.0
2.11.38.	"	"	0.5	"	6	"	" 4	"	- 2.0

Total 19.0 oz. of Tincture of Crataegus in 10 days.

This was a case of oldstanding myocardial degeneration with secondary effects due to congestion and oedema. The kidneys were to some extent affected and there was a moderate degree of hypertension. The kidney lesion may have been the primary one but seems scarcely severe enough for that. Ultimately auricular fibrillation complicated the picture and there was a degree of decompensation of the enlarged heart. Dyspnoea became severe. At this stage large doses of tincture of Crataegus in water were given with the following effects.

The pulse rate fell from 110 beats per minute to


Graph VIII.
Dosage.
Pulse Deficit.
Pulse Deficit in beats/min.
Total Cantoague in ʒ. Tr. by mouth.
Time in Days.
1
2
3
4
5
6
7
8
9
10.
10.
20.
30.
4
8
12.
16.

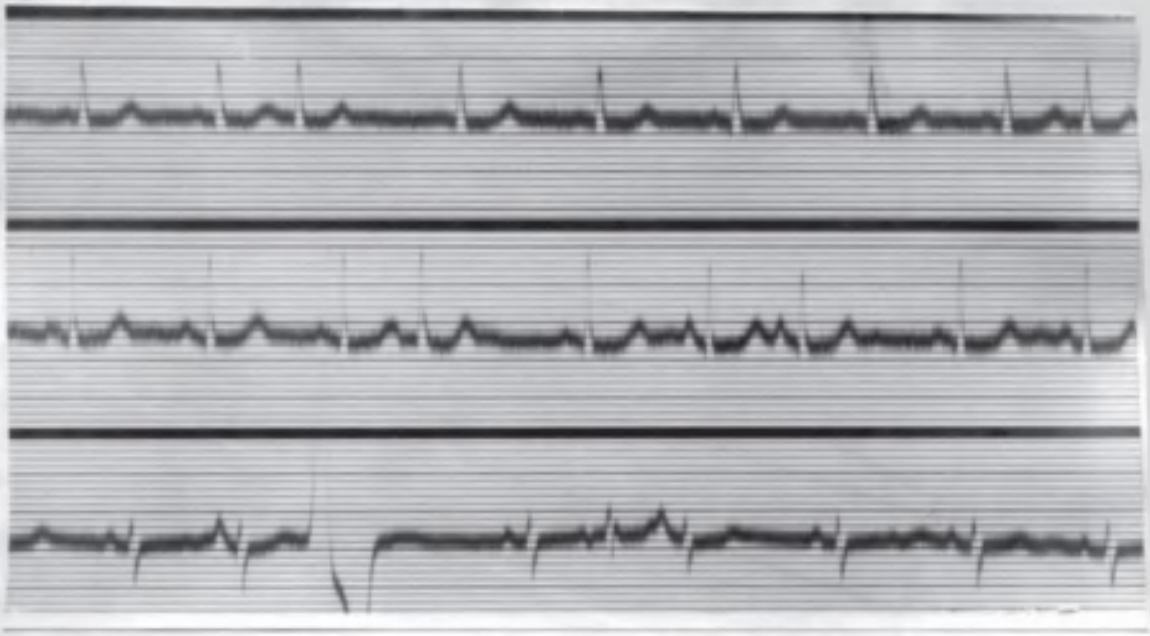


FIG. 32. Electrocardiogram of M.J.
Auricular Fibrillation.

90 beats per minute in 10 days. This fall might or might not be resultant upon exposition of the drug. The condition of auricular fibrillation per se was unaffected. The pulse deficit fell from 28 per minute to zero in six days. (see ~~Fig.~~ Graph VIII.). Ascites was less, the liver became less tender, oedema of the legs cleared up and icterus was gone in 8 days.

The blood pressure fell from 145/84 mm. Hg. to 116/75 mm. Hg. in five days and returned to its previous figure on giving Digitalis.

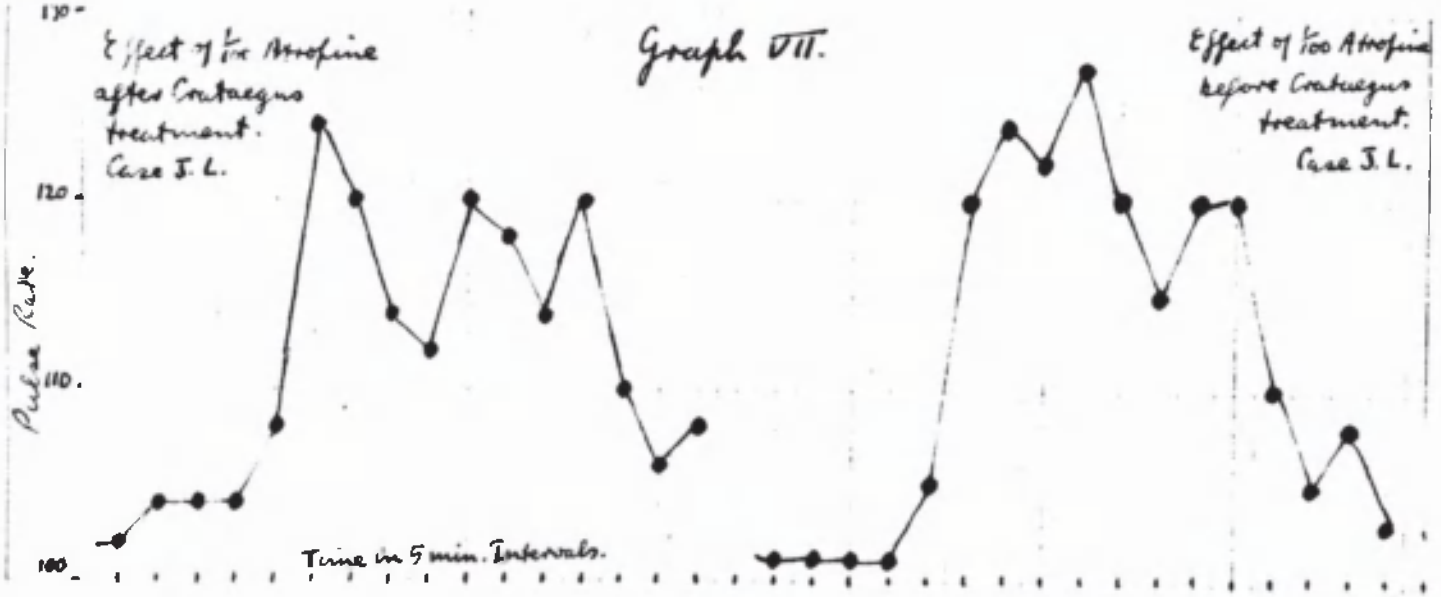
The water balance, previously unfavourable, was temporarily improved, but relapsed again. Digitalis had a similar effect.

Case. 2. M.J., female, aet. 76 years.

A case, not examined in such detail, of long standing auricular fibrillation with no oedema or congestion. The electrocardiogram is seen in Fig. 32.

There was no improvement of the cardiac condition under Crataegus.

From a consideration of these cases it would seem that tincture of Crataegus in extremely large doses by mouth may slow the pulse to a variable degree, undoubtedly strengthens the pulse as shown by the removal of pulse deficit, and may by this means promote the removal of oedema. This action on the pulse rate is inhibited by atropine, and in this way the clinical and therapeutic action of Crataegus is similar to that of Digitalis. This effect is shown in ~~Fig.~~ Graph VII.


Graph VII.
Effect of 1/100 Atropine after Crataegus treatment. Case J. L.
Effect of 1/100 Atropine before Crataegus treatment. Case J. L.
Pulse Rate.
130
120
110
100
Time in 5 min. Intervals.

The condition of auricular fibrillation is unaffected by tincture of Crataegus: the drug lowers the blood pressure sharply.

(b) Mitral Stenosis with Decompensation.

Case. 1. M.B., female, aet.38 years. Admitted 16.10.38.

Complaint. Palpitation of 5 years duration. Dyspnoea of 10 months duration, and oedema of the legs of 6 weeks duration.

History. Patient had 6 children. No history of Scarlet fever, diphtheria or rheumatism. Nephritis during pregnancy in 1922. Severe blood loss from ante-partum haemorrhage 3.5 years ago, dyspnoea after that and oedema lately. Orthopnoea recent.

Examination.. Pale, orthopnoeic woman with no cyanosis. Oedema of the legs and sacral region is present. Malar flush is marked, and distension of the veins of the neck.

Cardio-vascular System. Pulse 100 per minute, of low tension, and regular. Apex beat in the 5 th. interspace 2.5 inches from the mid line. Apical thrust diffuse, presystolic thrill. Presystolic and diastolic bruit at apex, first sound abrupt.

Respiratory System. Small effusion at left base with Grocco's triangle.

Alimentary System. Dental caries. Liver palpable 2 inches below costal margin.

Central Nervous System. N.A.D.

Blood. Hb. 77%. R.b.c. 3.79 mils. W.R. - ve.
C.I. 0.92 w.b.c. 11,800.

Case history.

19.10.38. Oedema bad. Feeling better from rest. No dyspnoea.

24.10.38. Cyanosis appeared. Urinary output poor. Urine normal. Pulse rate 100 per minute. Treatment begun.

25.10.38. B.Pr. 110/70 mm. Hg. Liver 12 cm., palpable for 2 cm., tender.

26.10.38. Nausea, tongue furred, dyspnoea at night. Oedema advancing.

27.10.38. Oedema worse. Medicine said to cause sleepiness. B.Pr. 100/70 mm. Hg. Pulse 88 per minute. Ascites showing in abdomen.

28.10.38. Treatment stopped. Digitalis substituted.

30.10.38. Better. No dyspnoea, pulse slowed and urinary output increased.

3.11.38. Bradycardia and sickness. Digitalis stopped. Pulse strong. Oedema gone. Dyspnoea gone. B.Pr. 110/75.

Water Balance.	In.	Out.		In.	Out.
18.10.38.	20 oz.	14 oz.	26.10.38.	16 oz.	20 oz.
19.10.38.	25 "	24 "	27.10.38.	17 "	22 "
20.10.38.	12 "	12 "	28.10.38.	18 "	22 "
21.10.38.	18 "	16 "	29.10.38.	17 "	20 "
22.10.38.	15 "	24 "	30.10.38.	20 "	22 "
23.10.38.	20 "	16 "	31.10.38.	20 "	49 "
24.10.38.	17 "	27 "	1. 11.38.	20 "	64 "
25.10.38.	15 "	25 "	2. 11.38.	18 "	48 "


Case M.B.
Tr. Crataegus begun.
Graph IX
Tr. Crataegus stopped.–
Digitalis begun.
Heart Rate in beats/min.
130.
110.
90.
70.
50.
1
2
3
4
5
6
7
8
9
10.
11.
12.
13.
Time in Days.

Treatment.

24.10.38. 0.5 oz. 2 doses. Total.

25.10.38. 0.5 " 4 " 1.0 oz.

26.10.38. 0.5 " 4 " 2.0 "

26.10.38. 0.5 " 4 " 2.0 "

27.10.38. 0.5 " 4 " 2.0 "

28.10.38. 0.5 " 2 " 1.0 "

Total 8 ozs. of tincture of Crataegus.

28.10.38. Tab.Dig.Fol. gr.2. 6 hrly. 3 dose. Total.

29.10.38. " " " 2 6 " 3 "

30.10.38. " " " 2 6 " 3 "

31.10.38. " " " 2 6 " 3 "

1. 11.38. " " " 2 6 " 3 "

2. 11.38. " " " 2 6 " 3 "

3. 11.38. Stopped.

Total. 34 grains. of Tab. Digitalis Folium.

This case was one of temporary decompensation with dyspnoea and oedema, in a young woman. Tincture of Crataegus 8 ozs. in four days reduced the pulse rate slightly, lowered the blood pressure somewhat and perhaps promoted sleep, but failed to check the advancing oedema and may even have promoted the same. Digitalis speedily induced bradycardia and diuresis. Graph IX.

From a consideration of these cases, few as they are, it seems proven that tincture of Crataegus has no beneficial effect on oedema of cardiac origin and but little power to slow the pulse rate. It may promote a feeling of wellbeing in patients with cardiac weakness and

even strengthen the heart beat to some extent but is in no way comparable with Digitalis in this respect. Its therapeutic value, if any, is not as a substitute for Digitalis.

(c) Hypertension.

The efficacy of tincture of Crataegus in cases of hypertension has been particularly recommended by Renon (1915). As a sedative and hypotensive in cases of arterio-sclerosis with hypertension it was given in doses of 5 minims once or twice a day over a long period. Martini (1932) showed that Crataegus given intravenously lowered the carotid blood pressure in dogs and this action has been confirmed on other mammals in the course of the present work. Furthermore it has been seen that the blood pressure was lowered in the cases already reviewed, especially in the case with a degree of hypertension, on administration of tincture of Crataegus. It was accordingly decided to examine the effects of moderate doses of tincture of Crataegus oxyacantha upon the recorded blood pressure in various cases of hypertension.

Case Histories.

Type. A. ARTERIOSCLEROSIS with CHRONIC HYPERTENSION.

Case. 1. W.T., male, aet. 82 years.

Diagnosis. Cerebral hemmorrhage.

History. Fell down 2 years ago. Since paralysed on right side.

Examination. Fairly comfortable, well nourished old man, with no cyanosis, oedema or jaundice.

Central Nervous System. Pupils small and react in a sluggish manner. Absent knee and ankle jerks, on right side. Extensor plantar response on right side: no clonus.

Cardio-vascular System. Area of cardiac dulness enlarged to the left. Pulse irregular, vessels palpable. B.Pr 180/75 mm. Hg.

Respiratory System. Moist rhales both bases.

Alimentary System. Dental caries. Haemorrhoids and chronic constipation.

W.R. - ve. Urine normal.

Case 2.

A.S., male, aet. 71 years.

Diagnosis. Cerebral haemorrhage and senile dementia.

History. Nil.

Examination. Poorly nourished decrepit individual. No cyanosis, oedema or jaundice.

Central Nervous System. Spasticity of both legs, with exaggerated reflexes, clonus, and extensor plantar responses.

Cardio-vascular System. Pulse regular, tension high, vessels palpable. Heart enlarged to left. Sounds pure. B.Pr. 195/115 mm. Hg.

Respiratory System. Emphysema of both lungs. Moist rhales

Alimentary System. Dental caries.

W.R. - ve. Urine normal.

Case 3.

P.S., male, aet 64 years.

Diagnosis. Arterioeclerosis and senile dementia.

History. Nil.

Examination. Well-preserved old man: mentally very weak. No cyanosis, oedema or jaundice.

Cardio-vascular System. Pulse palpable, irregular rhythm, palpable vessels, high tension. Area of cardiac dulness enlarged to left. Soft aortic systolic bruit. B.Pr. 240/120 mm. Hg.

Respiratory System. Bases of lungs waterlogged. Cough troublesome.

Urine normal. W.R. - ve.

Case 4.

M.J., female, aet. 76 years.

Diagnosis. Auricular fibrillation. Arterio-sclerosis.

History. Vertigo, headache, weakness during past few years.

Examination. Old, poorly nourished woman, with no cyanosis, oedema, icterus or dyspnoea.

Cardio-vascular System. Pulse irregular, tension high, vessels palpable. Area of cardiac dulness increased to the left. Apex beat diffuse and heaving. Second aortic sound accentuated. B.Pr. 194/90 mm.Hg. Electrocardiogram typical of auricular fibrillation.

Urine normal. W.R. - ve. X-ray calcified arteries and enlarged heart.

Otherwise N.A.D.

Case 5.

M.N., female, aet. 67 years.

Diagnosis. Cerebral thrombosis.

History. Haematemesis 5 years ago. Loss of power of right hand, right leg and of speech progressively in last year.

Examination. Obese woman with spastic paralysis of right leg and paresis of right arm and right side of face with impairment of speech.

Central Nervous System. Extensor plantar response on right side with ankle clonus. Tongue deviates to the right side. Exaggerated reflexes on right side of arm and leg.

Cardio-vascular System. Pulse regular, high tension, palpable vessels. Area of cardiac dulness enlarged to the left. Soft systolic bruit at apex and aortic area. Second aortic sound slapping in character. B.Pr. 218/100. Urine normal. Spleen enlarged. W.R. - ve.

Case 6.

J.F., female, aet. 65 years.

Diagnosis. Cerebral haemorrhage.

History. Paralysis of both arms and legs of 14 days duration.

Examination. Well nourished. No cyanosis, jaundice or oedema.

Central Nervous System. Arm reflexes exaggerated, mental retardation marked, bilateral papilloedema, cerebellar loss of control.

Cardio-vascular System. Pulse rapid, high tension, regular. Area of cardiac dulness enlarged to left. Soft systolic bruit at tricuspid area. B.Pr. 235/110 mm.Hg

W.R. - ve. Cerebro-spinal fluid under pressure.

TYPE B. CHRONIC NEPHRITIS WITH HYPERTENSION.

Case 7.

J.T., male, aet. 67 years.

Diagnosis. Chronic nephritis and cardiac asthma.

History. Winter cough of many years duration, with increasing dyspnoea, cardiac pain, asthmatic attacks and weakness. Death followed upon perforation of a gastric ulcer.

Examination. Ill nourished man. Cyanosis but no oedema.

Cardio-vascular System. Pulse regular, high tension, vessels palpable. Area of cardiac dulness enlarged to the left. Sounds pure but remote. B.Pr. 200/120 mm. Hg.

Respiratory System. Dyspnoea, cough, emphysema, moist rhales at bases.

Alimentary System. Dental caries. Slight ascites.

Urine. Sp. gr. 1014. Albumin, a trace. A few granular casts.

Blood urea 44 mgm.%. W.R. - ve.

Post mortem examination. Chronic interstitial nephritis. Perforated chronic ulcer on lesser curvature of stomach.

Case 8.

A.C., male, aet. 63 years.

Diagnosis. Cerebral haemorrhage and chronic nephritis.

History. Sudden paralysis and loss of speech six months ago.

Examination. Well nourished man, pale mucous membranes.

Central Nervous System. Aphasia. Doubly incontinent.

Right facial paralysis, increased arm reflexes, right plantar response extensor, no clonus.

Cardio-vascular System. Pulse regular, high tension. Area of cardiac dulness enlarged to left. Sounds pure. B.Pr. 215/100 mm. of Hg.

Blood urea 43 mgm.%. W.R. - ve.

Urine has a trace of albumin, and a few casts.

Case 9.

J.McG., male, aet. 75 year.

Diagnosis. Cerebral thrombosis.

History. Attacks of cramp on exertion for six years. Cerbration impaired and legs stiff for three years. Death in sleep.

Examination. Large florid man with cyanosis of the face, and varices on the legs.

Central Nervous System. Arm and leg reflexes exaggerated. Ankle clonus and extensor plantar response on right side.

Cardio-vascular System. Pulse regular, high tension, vessels palpable. Area of cardiac dulness enlarged to the left. Soft aortic systolic bruit. B.Pr. 185/80 mm.

Blood urea 41 mgm.%. W.R. - ve.

Pus and granular casts in urine.

Post mortem examination. Chronic interstitial nephritis.

Case 10.

M.W., female, aet. 68 years.

Diagnosis. Chronic nephritis and cholecystitis.

History. Weakness and sickness of one year duration. Dyspnoea for many months. Frequency of urine and

oedema of ankles increasing.

Examination. Large woman with cyanosis, icterus and oedema of ankles..

Cardio-vascular System. Pulse slow and irregular, high tension; area of cardiac dulness enlarged to the left. Sounds poor. B.Pr. 200/60 mm. Hg.

Alimentary System. Liver palpable, gall bladder tender and enlarged.

Urine has pus and blood in it. W.R. - ve.

Blood urea 71 mgm.%.

X-ray shows vesical calculus and cholecystitis.

These ten cases of hypertension of various aetiological types were put on a dosage of one dram of tincture of Crataegus oxyacantha three times per day in water and the blood pressure registered daily with the same Beaumanometer. There were no ill effects from this treatment in the majority of the cases. Case No. 7 died suddenly from perforation of a chronic gastric ulcer on the lesser curvature of the stomach. His symptoms were vague and he complained of some abdominal pain unrelated to meals and unrelieved by alkali. It is possible that the tincture of Crataegus aggravated this condition. Case No. 9 died after an alarming drop in blood pressure. It is possible that overdosage with Crataegus may have aggravated his uraemic condition.

The effect of one dram of tincture of crataegus t.d.s. ex aq. was as follows:-

Case 1. B.Pr. 190/105 mm Hg. dropped to 150/90 mm.

in six days, remained there during exhibition of the drug and returned to normal in ten days.

Case 2. B.Pr. 195/100 mm.Hg. dropped to 150/80 mm. in 17 days, remained there during exhibition of the drug and returned to normal gradually.

Case 3. B.Pr. 200/110 mm. Hg. dropped to 140/80 mm. in 17 days and remained there during exhibition of the drug.

Case. 4. B.Pr. 170/87 mm. Hg. dropped to 140/70 mm. in 17 days and remained there during exhibition of the drug.

Case. 5. B.Pr. 170/100 mm. Hg. dropped to 150/85 mm. in 7 days and remained there during exhibition of the drug.

Case. 6. B.Pr. 240/120 mm. Hg. dropped to 170/90 mm. in 12 days and remained there during exhibition of the drug, returning to normal gradually.

Case. 7. B.Pr. 200/115 mm. Hg. dropped to 175/100 mm. in 22 days, remained there during exhibition of the drug and returned to normal in 10 days.

Case 8. B.Pr. 210/110 mm. Hg. dropped to 160/90 mm. in 17 days and remained there during exhibition of the drug.

Case. 9. B.Pr. 195/110 mm. Hg. dropped to 110/70 mm. in 10 days and remained there despite stoppage of the drug. Death ensued on the 15 th. day.

Case 10. B.Pr. 180/70 mm. Hg. dropped to 150/55 mm. in 15 days, remained there during exhibition of the

drug and slowly rose again.

These changes in the blood pressure of patients are illustrated by the accompanying series of blood pressure charts.

A consideration of the foregoing records, indicates that tincture of Crataegus oxyacantha taken by mouth in doses of one dram three times per day in water has a powerful effect in lowering the blood pressure.

This action might usefully be employed in a less drastic manner in cases of hypertension, whether due to arterio-sclerosis or following upon chronic renal lesions, in order to avoid cerebral haemorrhage and thrombosis, and perhaps the onset of uraemic symptoms of a cerebral nature. For this purpose prolonged use of a smaller dose is suggested eg. 5 minims b.d. ex aq.

In hypertensive crises, such as in essential hypertension, larger doses may be used. There is relatively little effect on the normal blood pressure, as is shown in the case discussed which had mitral stenosis and decompensation. The use of tincture of Crataegus for this purpose is in no way curative but might be of great prophylactic value.

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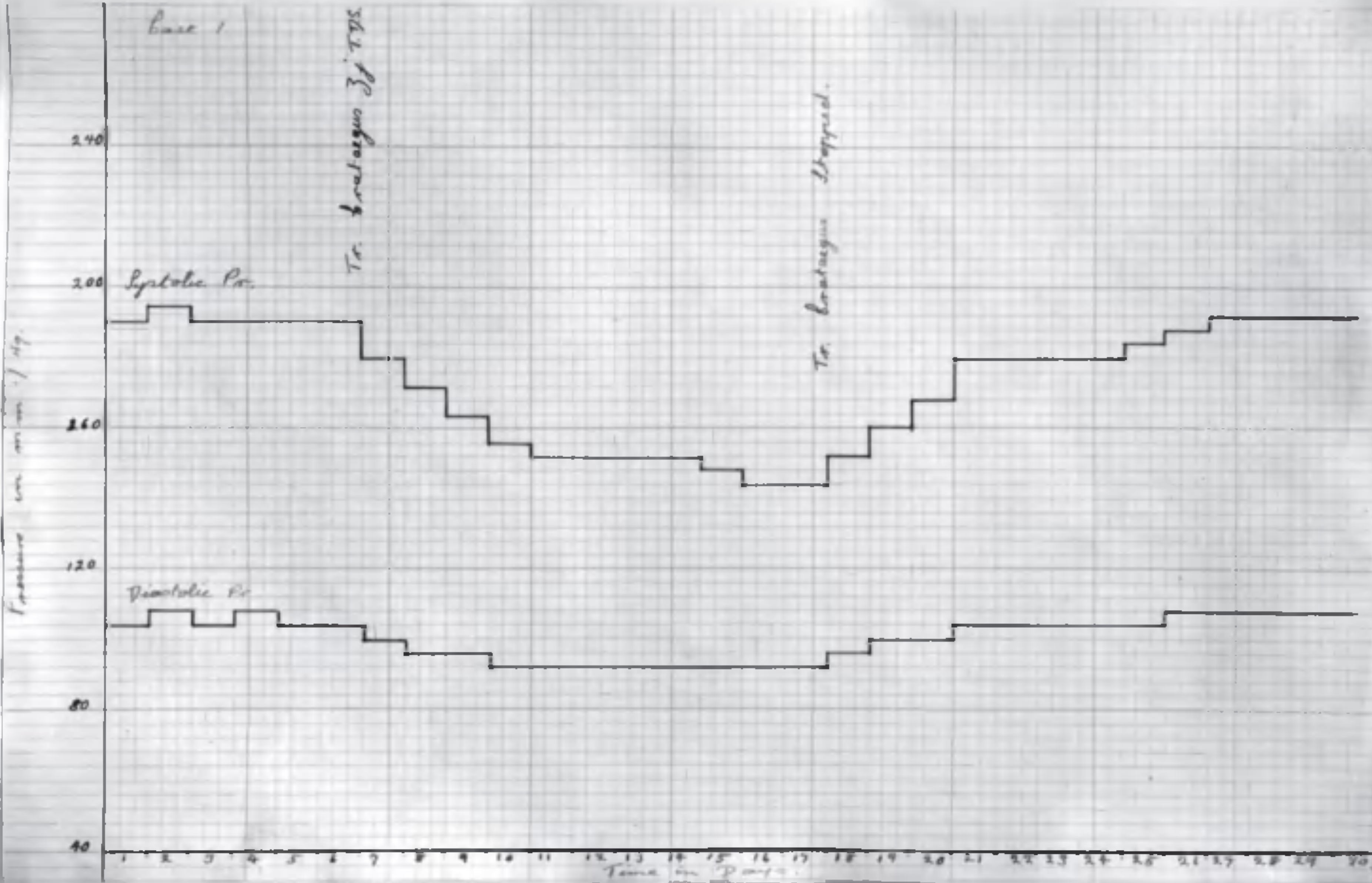
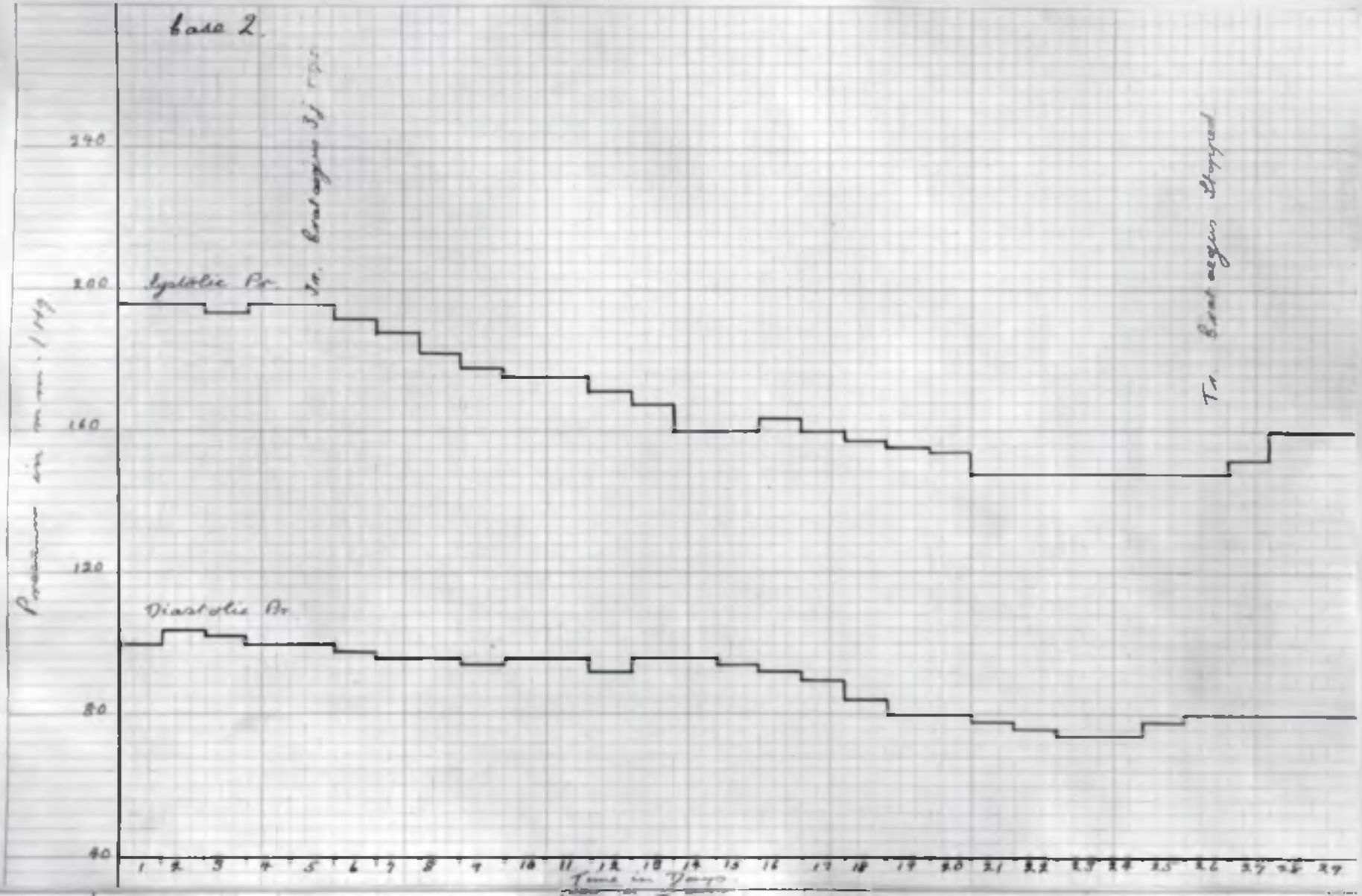
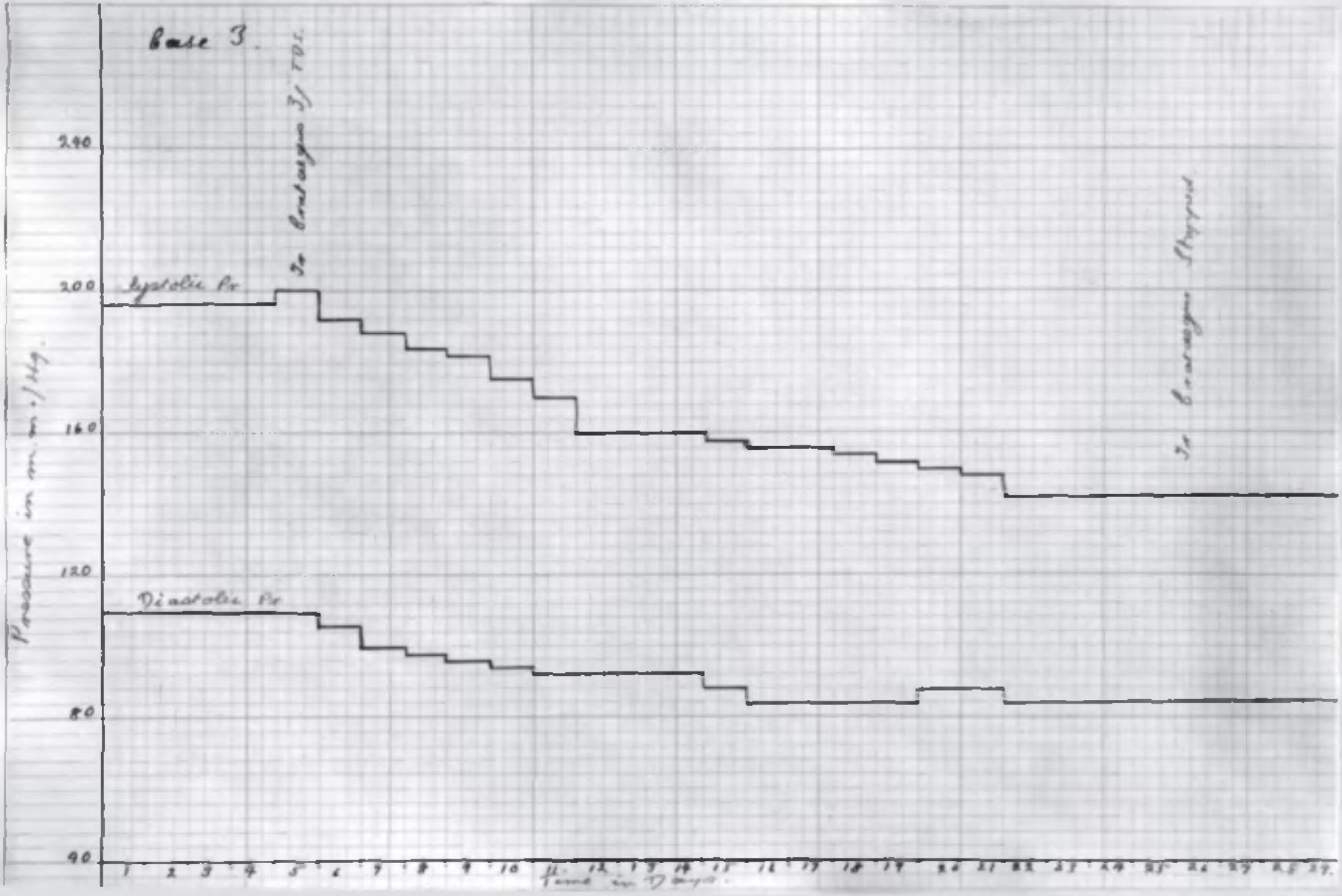
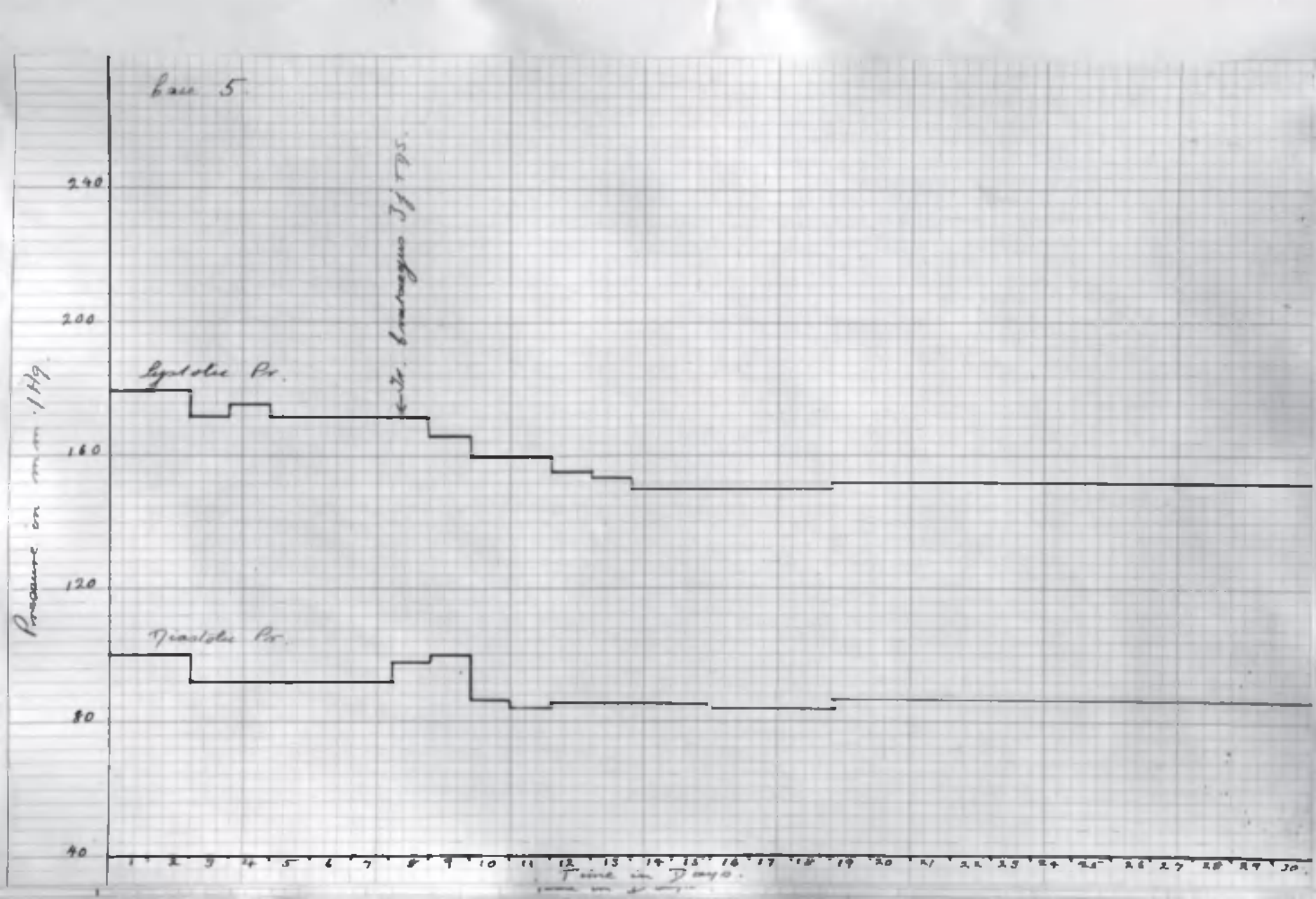
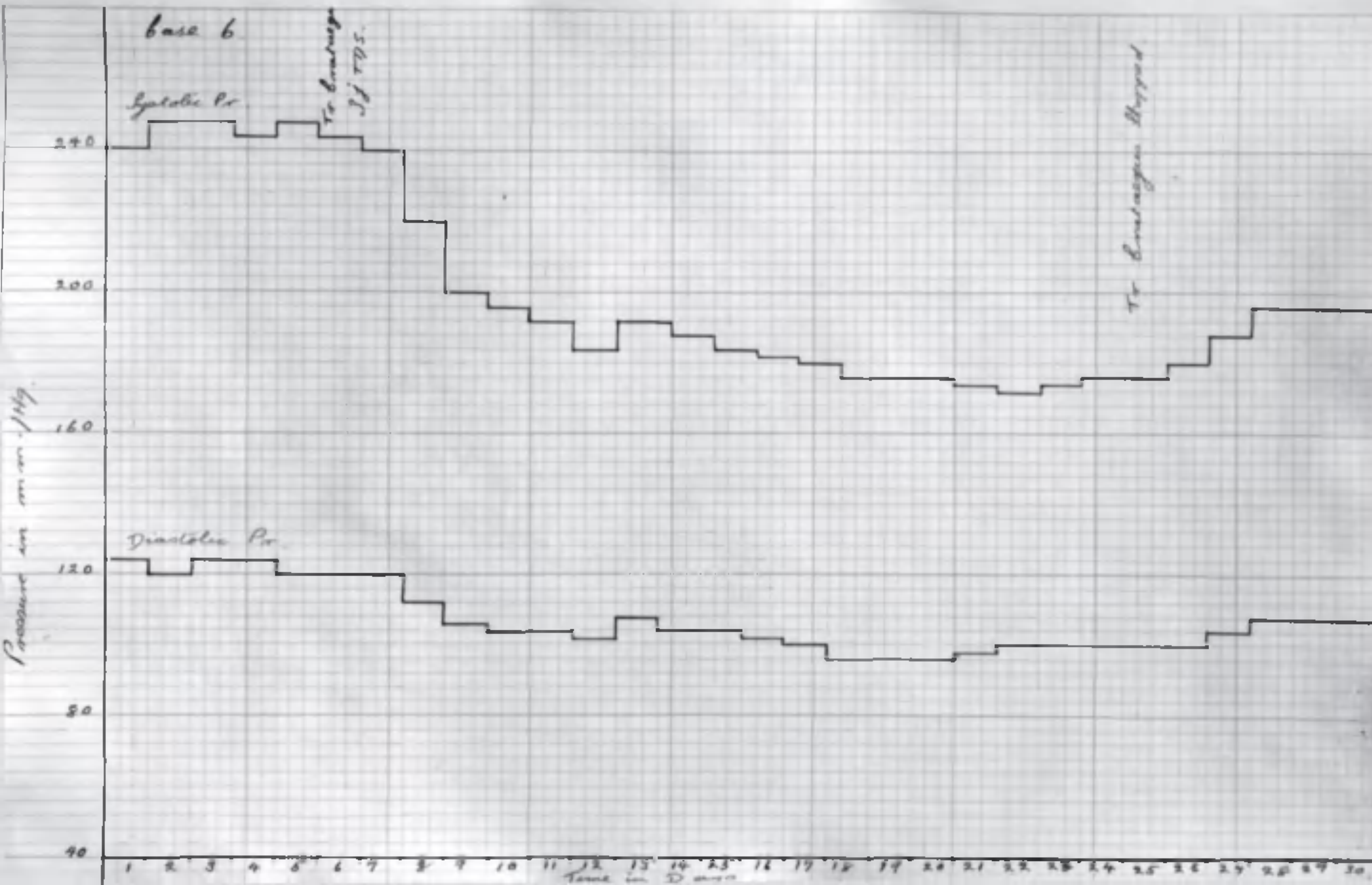

Case 1
Tr. Crataegus 3j t.i.d.
Tr. Crataegus Stopped.
Systolic Pr.
Diastolic Pr.
240
200
160
120
80
40
Pressure in mm / Hg.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Time in Days

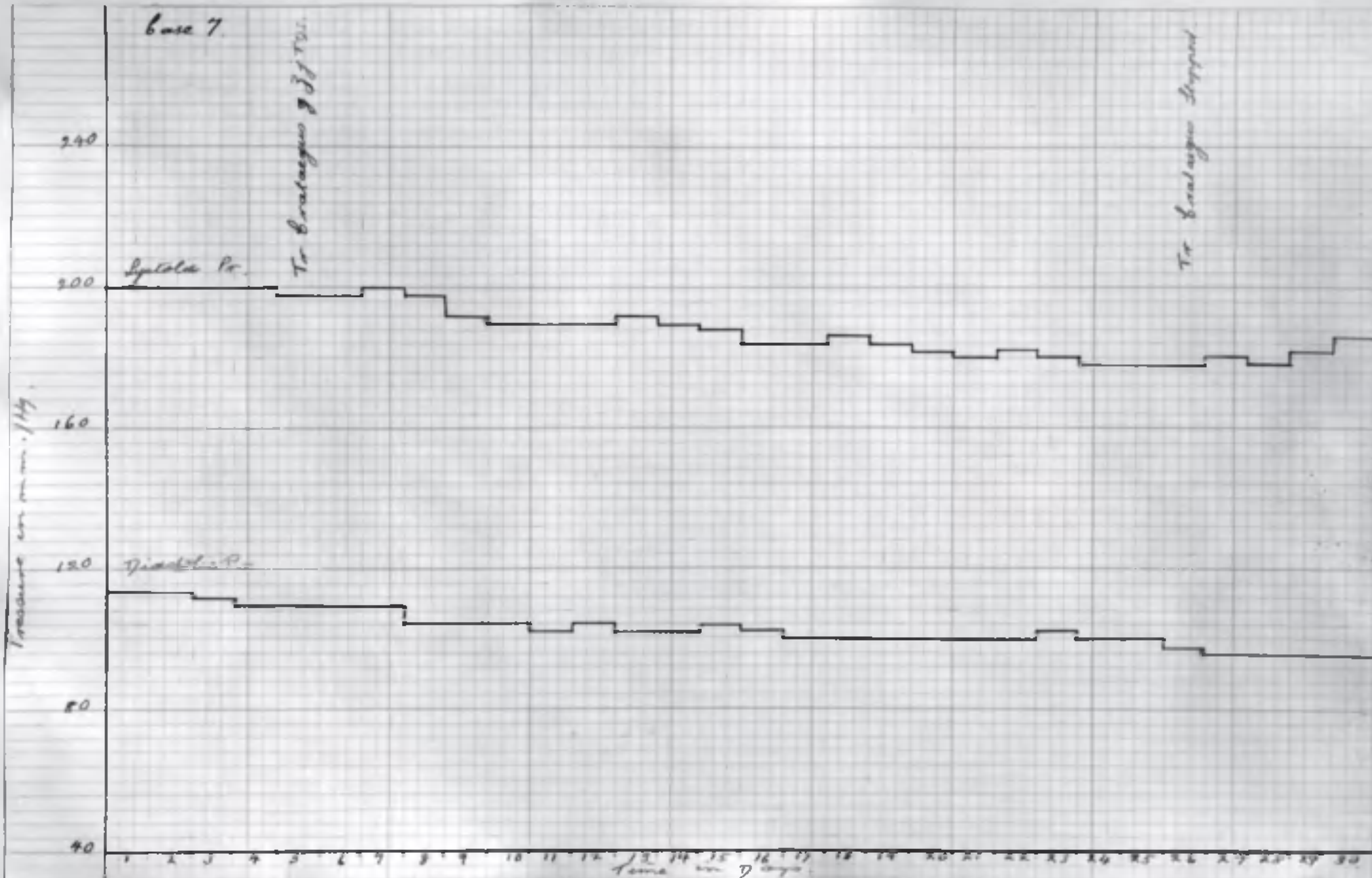

base 2.
240
200
160
120
80
40
Systolic Pr.
Diastolic Pr.
Pressure in m.m. of Hg.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Time in Days

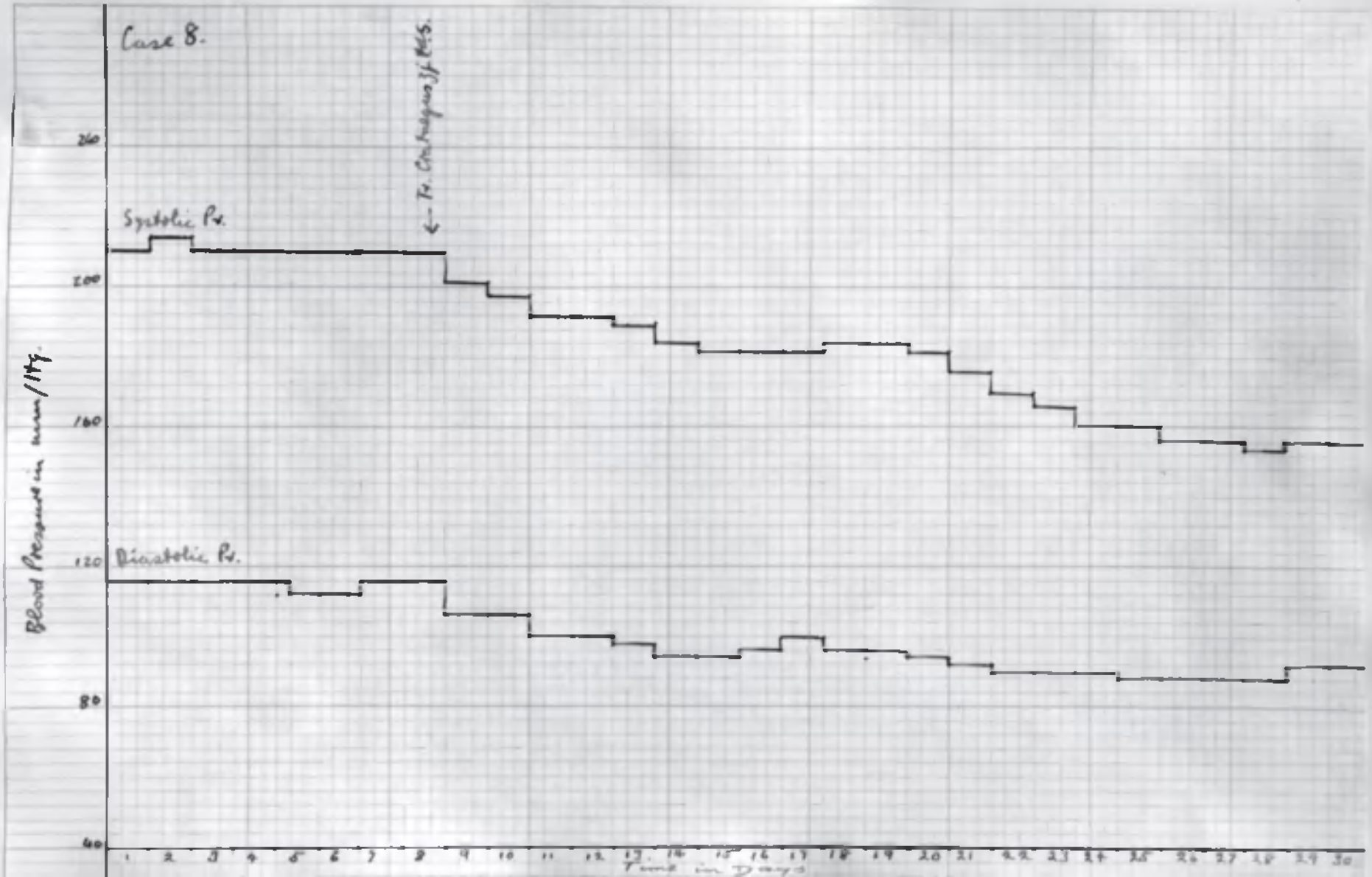

Base 3.
Systolic Pr
Diastolic Pr
Pressure in m.m. / Hg.
240
200
160
120
80
40
Time in Days.

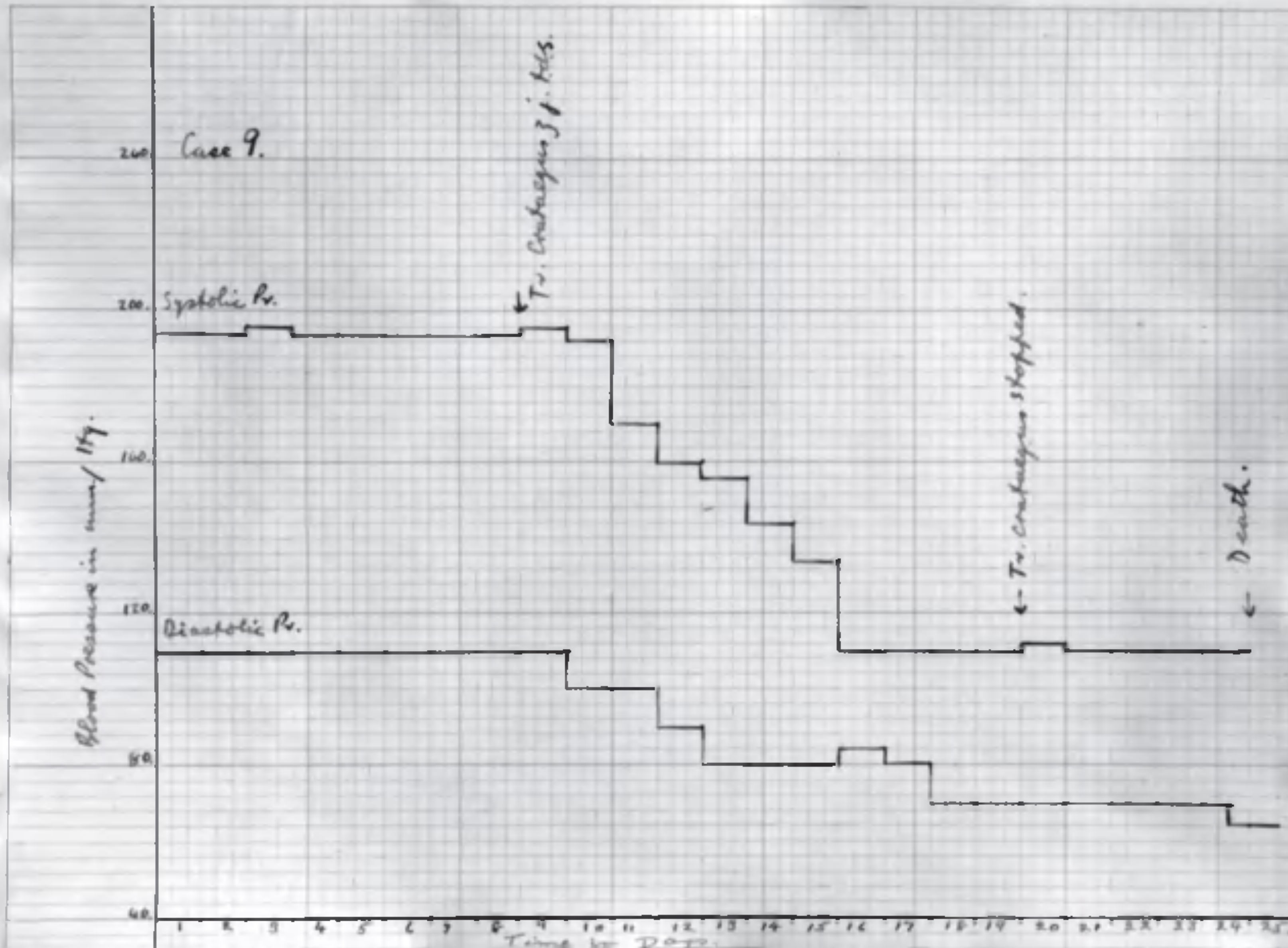
Blood Pressure in mm /Hg.
240
200
160
120
80
60
Case 4.
Systolic Pressure.
← Tr. Cardiaque diastolique.
← Tr. Cardiaque systolique
Time in Minutes.

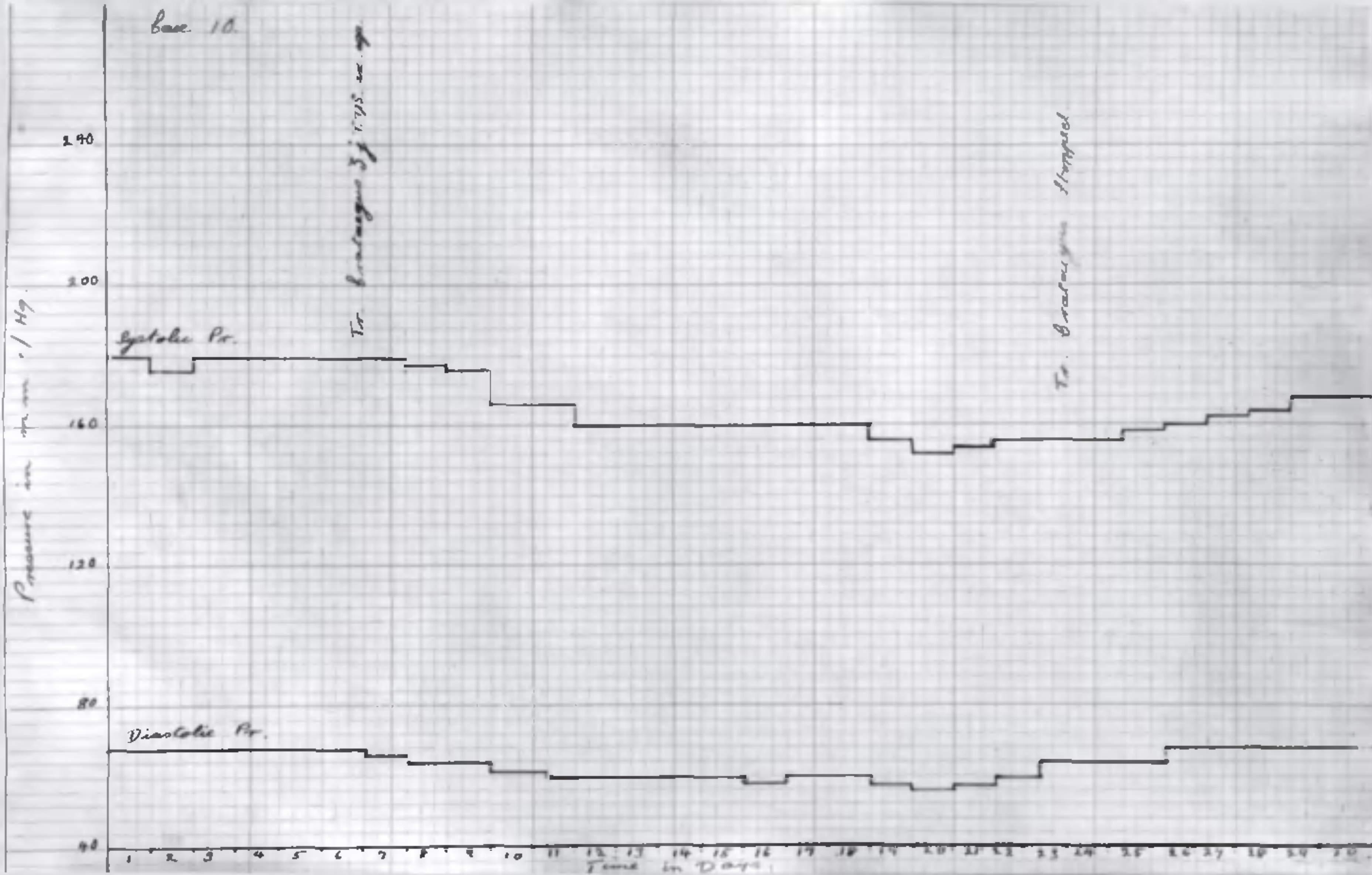

Case 5.
240
200
160
120
80
40
Pressure in mm. Hg.
Systolic Pr.
Diastolic Pr.
Intravenous Injections.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time in Days.


Case 6
Systolic Pr.
Diastolic Pr.
Pressure in mm/Hg
240
200
160
120
80
40
Time in Days


Case 7.
Tr. Eralague 3 ℥j t.d.
Tr. Eralague Stopped
Systolic Pr.
Diastolic Pr.
240
200
160
120
80
40
Pressure in mm. Hg
Time in Days


Case 8.
← Tr. Cerebrospinal Mts.
Systolic Pr.
Diastolic Pr.
Blood Pressure in mm/Hg.
260
200
160
120
80
40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time in Days


Case 9.
Systolic Pr.
Diastolic Pr.
Tr. Crataegus ʒ j. tds.
Tr. Crataegus stopped.
Death.
240.
200.
160.
120.
80.
40.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Blood Pressure in mm/Hg.


Case 10.
Systolic Pr.
Diastolic Pr.
Pressure in mm / Hg.
240
200
160
120
80
40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time in Days

DISCUSSION.

The common Hawthorn (Crataegus oxyacantha) contains a specific active principle which is of the nature of Digitalis. This statement may be made with a fair degree of confidence when it is remembered that the actions of Digitalis and Crataegus are so alike.

The actions of Digitalis are as follows:-
External. Preparations of Digitalis if injected may cause abscesses.
Internal. Digitalis produces emesis and diarrhoea; it reverses the motor gradient of the bowel. In mammals the heart beat is slowed, the diastolic pause prolonged, spontaneity increased and heart block induced. In frogs the effects are complicated by the onset of contracture. The vagal centre in the medulla of mammals is stimulated. In the tortoise the isolated heart is inhibited. The blood pressure in mammals is raised by moderate doses of Digitalis, the coronary and pulmonary arteries constricted: in therapeutic doses in man the pressure is unaffected. Digitalis is a diuretic in health and in disease, markedly so in cases of cardiac oedema. Large doses of Digitalis stimulate the respiratory centre; the drug is oxytocic to the uterus and stimulates the medulla of mammals while inhibiting the spinal cord of frogs. Therapeutically Digitalis is of extreme value in tachycardia with irregularity, with or without decompensation. It is specific in cardiac failure from auricular fibrillation, with oedema and dyspnoea.

The actions of Crataegus oxyacantha are as follows:-
External. On injection tincture of Crataegus causes abscesses.
Internal. Crataegus produces emesis; it may produce diarrhoea; it tends to reverse the motor gradient of the bowel. The gastric motility is increased, as is the case with Digitalis. In mammals the heart beat is slowed, the diastolic pause prolonged, spontaneity increased and heart block induced. The vagal centre in the medulla is stimulated, the early effects being inhibited by atropine. After inhibition of the parasympathetic nerve supply to the heart cardiac inhibition may still be induced by Crataegus. In frogs the effects are complicated by the onset of contracture but are reversible in the early stages by washing with saline. There is a tendency to early vagal inhibition with spontaneous recovery which is followed by a prolonged phase of augmentation. In the tortoise the isolated heart is inhibited in a manner identical with the action of strophanthin and Digitalis. The isolated mammalian heart exhibits interference with the conducting system, while the avian heart reacts to Crataegus in a way very similar to the action on the mammalian heart. The blood pressure in mammals is temporarily raised by moderate doses of Crataegus, followed by a prolonged fall in blood pressure. In this action lies the most striking pharmacological point of difference between Digitalis and Crataegus oxyacantha. The coronary

arteries are temporarily constricted, then dilated: the pulmonary arteries are constricted. In therapeutic doses in man there is a prolonged fall in blood pressure which is maintained so long as the exhibition of the drug is continued, and which is restored after discontinuation of the drug. Crataegus is an anti-diuretic in health and disease. It has little or no effect on cardiac oedema. Crataegus inhibits the respiratory centr of mammals after a short preliminary stimulation, and is oxytocic to the uterus in vitro. It stimulates the medulla of mammals and inhibits the spinal cord of frogs. Therapeutically Crataegus has only a slight beneficial effect on tachycardia with irregularity, and does little of benefit to cases of auricular fibrillation with dyspnoea and oedema. Its field of activity is in hypertension.

The majority of these pharmacological actions agree closely with the actions of Digitalis. The main differences are those of degree rather than of kind. They may be summarised as follows:-

1. The depressant action on the mammalian blood pressure and the action in dilating the perfused vessels of the frog.
2. The therapeutic action in lowering the blood pressure in man in doses which do not affect the heart.
3. The toxic effect on the respiratory centre of mammals.
4. The toxic effect on the liver of mammals.

5. The degree of action on the vagal apparatus in the frog heart, and the persistance of action on the auricle of the denervated mammalian heart under atropine.

When the effect on the vessels is considered it is seen that small doses of Crataegus have the same effect as larger doses of Digitalis. Neverthless it is curious that small doses of Crataegus dilate the coronary vessels temporarily in the isolated mammalian heart and constrict the frog's systemic vessels, while large doses have the opposite effect, so that in one case large doses of Crataegus simulate the action of Digitalis and in the other case are opposed to it. The therapeutic action is in keeping with the pharmacological action on the vessels but seems to be of an extraordinary potency in relation to the dosage.

The toxic effects on the respiratory centre and liver are not unopposed to the actions of Digitalis but are more severe. It is noteworthy that comparable doses of Crataegus and Digitalis affected the liver in the former case and the heart in the latter case. In both cases necrosis(toxic) was produced.

The degree and manner of inhibition of the heart under these two drugs under experimental conditions is a matter of little difference, but the persistance of the diastolic inhibition of the mammalian and avian hearts after atropinisation, with a strong tendency to spontaneous recovery, is extraordinary. The mode of action of Crataegus may be more on the muscle fibre

of the heart than on the medullary centres than is the case with Digitalis, since there are undoubted differences in the chemical constitution of the pure principles. It is noteworthy that none of the common methods of extraction of Digitalis glycosides is successful in isolating the principle of Crataegus oxyacantha. The lack of potency of the tincture as assayed biologically in comparison with Standard Tincture of Digitalis is of little importance since the therapeutic action of Crataegus in heart disease is so poor. If this preparation were to be used to any extent as a means of reducing blood pressure in cases of hypertension it would be necessary to devise a new standard and method of biological assay, as for instance a unit defined as the quantity of tincture of Crataegus oxyacantha which reduces the carotid blood pressure in a cat of 2-2.5 kilos weight by 40 mm. Hg. pressure within one minute of injection in saline into the jugular vein, the cat being anaesthetised by intramuscular injection of nembutal 0.5 gr. per kilo of weight.

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SUMMARY.

1. A historical survey of the literature of Crataegus oxyacantha is given, and the origins and development of its use in medicine considered.

2. A historical survey of the literature of Digitalis and its allies is given, and the development of the investigation of the drug and its uses systematically cosidered.

3. The experimental work on Crataegus oxyacantha is next considered.

4. It is shown that the principle extracted from Crataegus oxyacantha by Baechler (1927) and called by him "crataegussäure" is very unlikely to be the specific active principle of Crataegus oxyacantha.

5. It is shown that the fruits of Crataegus oxyacantha contain no alkaloidal or glycosidal material. The contention of Wicke (1853) that the fruits of Crataegus oxyacantha contain a cyanogenetic glycoside is refuted.

6. Various methods of extraction were tried in an unsuccessful endeavour to isolate the active principle in a pure form.

7. It is shown that this principle is water soluble, readily oxidised, and present in the pulp of the fruits and the petals of the flowers but not in the seeds or the wood.

8. Certain tentative conclusions are drawn with regard to the pigments of the fruit skins.

9. The exact method of preparation of the galenicals

drake induces slowing of the heart with augmentation of the beat. This effect is inhibited by atropine sulphate.

20. In larger doses Crataegus oxyacantha is toxic to the avian heart, inducing auricular fibrillation, heart block and cardiac arrest from ventricular fibrillation.

21. These observations are supplemented by electrocardiographic records.

22. In the drake Digitalis primarily affects the conducting system of the heart, Crataegus the cardiac muscle.

23. The action of Crataegus oxyacantha on the intact mammalian heart is considered.

24. In the cat Crataegus oxyacantha given intravenously induces slowing of the heart rate and augmentation of the beat. This effect is inhibited by atropine.

25. Toxic doses give rise to extra-systoles, bradycardia, auricular fibrillation and cardiac arrest from ventricular fibrillation.

26. In the atropinised vagotomised cat intravenous Crataegus oxyacantha causes diastolic arrest of the auricle. This action must be a direct one on the muscle fibres.

27. In the perfused isolated rabbit heart Crataegus oxyacantha has an action similar to that on the frog heart.

28. Existing irregularities are removed.

29. Heart block is early induced.

30. The effect of Crataegus oxyacantha and [illegible] of

Digitalis on the electro-cardiogram of the cat and guinea pig is studied.

31. In the cat Crataegus oxyacantha given intravenously affects the conducting system chiefly and inhibits the auricle; Digitalis strengthens the auricle but affects conduction and contraction in the ventricle.

32. The normal heart rate and conduction and contraction times in the electro-cardiogram of the guinea pig is discussed.

33. Chronic poisoning with Crataegus oxyacantha in the guinea pig affects the auricle, the conduction rate, and the muscular contraction; Digitalis has similar effects.

34. Tincture of Crataegus oxyacantha given intravenously in the cat causes a temporary rise in blood pressure which is succeeded by a prolonged and progressive fall in blood pressure.

35. Infusion of Crataegus in high dilutions constricts the perfused blood vessels of the frog, in low dilutions it is an active vaso-dilator. Digitalis acts as a vaso-constrictor.

36. The technique of Hanzlik (1936) for demonstrating changes in the circulation in the rabbit's ear is reviewed and discussed.

37. The effect of Crataegus oxyacantha so measured is variable.

38. Crataegus oxyacantha constricts the isolated coronary arteries of the sheep, as does Digitalis.

39. Crataegus oxyacantha acts as a temporary dilator of the coronary circulation of the isolated mammalian heart, followed by marked vaso-constriction.

40. The findings of Martini (1932) are partially corroborated and partially refuted.

41. Crataegus oxyacantha constricts the pulmonary vessels.

42. Given intravenously Crataegus oxyacantha inhibits the respiratory centre of mammals. Temporary stimulation may occur.

43. In the bird this inhibition of respiration is not so marked.

44. Crataegus oxyacantha constricts the bronchi of mammals.

45. In the intact white rat Digitalis acts as a diuretic, Crataegus oxyacantha as an anti-diuretic.

46. Crataegus oxyacantha stimulates the medullary centres in the mammal and inhibits the spinal cord in the frog.

47. Crataegus oxyacantha in small doses is motor to the mammalian intestine in vitro; large doses inhibit it.

48. Crataegus oxyacantha increases gastric motility in vivo and inhibits the colon.

49. Crataegus oxyacantha is oxytocic to the pregnant guinea pig uterus in vitro, inhibitor in vivo.

50. A table of lethal doses for Crataegus tincture for various animal species is given.

51. Crataegus oxyacantha in repeated parenteral doses

is not generally toxic to the rat.

52. Crataegus oxyacantha in repeated subcutaneous doses is toxic to the guinea pig.

53. Necrosis of the liver is shown to have occurred in guinea pigs so treated.

54. There are no morphological changes in the hearts of guinea pigs so treated.

55. Results for the chemical assay of tincture of Crataegus oxyacantha by the method of Knudson and Dresbach (1923) are given. These are compared with two biological assays by the frog method and two biological assays by the cat method.

56. The results of clinical trial of tincture of Crataegus in cases of cardiac decompensation with mitral stenosis and with auricular fibrillation are given.

57. Tincture of Crataegus is in no way comparable with Digitalis as a therapeutic agent in such cases.

58. The results of clinical trial of tincture of Crataegus in 10 cases of hypertension are given.

59. Crataegus oxyacantha is a potent agent in reducing the blood pressure in such cases.

60. Its employment in cases of hypertension from arterio-sclerosis, chronic nephritis, and essential hyperpiesia is advocated.

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