REPORT

of

COMMITTEE ON EXAMINATION

This is to certify that we the undersigned, as a Committee of the Graduate School, have given Florence Defiel final oral examination for the degree of Master of Arts. We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

Dec. 3 1921

Wm A. Riley
Chairman

Arthur T. Henrici

Royal N. Chapman

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Florence Defiel for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

Wm. A. Riley
Chairman

Royal N. Chapman

[signature]

Nov. 23 1921

STUDIES ON THE SUPPOSED TOXIC QUALITIES OF CALENDRA GRANARIA, AND CERTAIN OTHER COLEOPTERA.

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

OF THE

UNIVERSITY OF MINNESOTA

BY

FLORENCE DEFIEL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE

DEGREE OF

MASTER OF ARTS

DECEMBER

1921

I. Introductiom.

In view of the fact that comparatively little work has been done in this country on vesicating insects, and also that there are extant many opinions concerning poisonous insects which have surprisingly little data as foundation, the subject of this paper was suggested by Dr. W. A. Riley as one worthy of study.

It is well known that the beetles belonging to the family Meloidae contain a substance which is highly toxic when ingested, and for which no antidote is known. This same substance, when applied to the body surface, is an active vesicant, and from time to time other beetles have been supposed to possess these same vesicating qualities. The granary weevil, Calendra granaria, has been mentioned in this connection, and there is a widespread belief that it may be used as a substitute for the officinal blister beetle, Cantharis vesicatoria. As far as has been determined, there is no experimental evidence in support of this belief.

That similar views were held regarding the Colorado potato beetle, Leptinotarsa decemlineata, may be readily observed if one examines the literature of economic entomology in the United States for the period 1865-1875. The beetles aroused a great deal of comment at that time, and were believed to contain a volatile poison capable of causing a severe or even fatal illness.

Since the properties attributed to both of these insects are actually possessed by the Meloidae, and a great deal of work has been published on this group, it is reasonable to suppose that a thorough search of this literature is the most expeditious manner in which to approach the question.

II. History of the Discovery of Cantharidin.

Physicians, toxicologists and entomologists have long been interested in the Meloidae or Cantharides as is evidenced by the interesting account of Moufet (1658), and by the reviews of ancient literature included in papers by Ferrer (1859), Beauregard (1890), Raillet (1895) and Chalmers and King (1917). As far back as Hippocrates in the fifth century B. C. we find the term "Cantharides" used to designate Coleoptera which were used as internal remedies in such diseases as jaundice, dropsy and apoplexy. Aristotle in the fourth century B.C. used the name Cantharis; and Dioscorides in the first century A.D. stated that the blister beetles with the yellow-banded fat body were more efficient than those all of one color.

A little later, in the first century A.D., Pliny advised their use as an emmenagogue and a diuretic in leprosy. He also mentioned for the first time their use as vesicating agents, a fact discovered by Archigenes, a contemporary of Celsus, or perhaps by Aretacus of Cappadocia. The doctors of that time recognized three varieties of vesicants; one plump and fat with yellow markings on the wings; the second, smaller, broad and hairy; while the third, and least efficient as medicine, is all of one color and lean bodied. They did not attempt, however, to designate them more specifically. Another interesting fact which Chalmers and King brought out is that different workers preferred different parts of the insect for blistering, some the mouth parts, some the feet, etc., indicating to us the general distribution of the substance in the insect body.

Galen, Vegece and Hierocles are other physicians who used beetles which they called Buprestids, but which were probably Meloids, because it is reported that they caused in cattle an inflammation which frequently brought on death. That their toxic qualities were well known is shown by the fact that there existed in Rome the "Law of Cornelia" which punished by death any person guilty of putting "Buprestids" in food or drink.

But the identity of the active principle remained unknown despite the prominent place which these insects held in the European pharmacopeia. It is characteristic of Middle Age scientists that the above mentioned writings alone served as bases for the accounts of Cantharides in the Tractus de Avibus of 1511 (Edition of the Ortus Sanitatis, published originally in 1491). The earlier German "Herbarius" was also derived from these ancient manuscripts, and no effort was made to add to the facts worked out by the ancient scientists.

In 1658 Moufet published a long treatise on Cantharides which will be considered later, and which stimulated a great deal of interest. This was almost immediately followed by the systematic work of Linnaeus, Fabricius, Latreille and Burmeister. In the latter part of the seventeenth century a chemist, Olaüs Borrichius of Copenhagen, experimented on the cause of vesication, and erroneously concluded that the hairs on the surface of the body were irritating. Then Leuwenhoek, Cockburne and Cartheuser attempted to analyze cantharides, but the only differentiation they were able to make was as follows: 1.5 grain gelatinous substance, 0.5 scruples resinous matter, and the rest clay, which was no nearer the solution than before. Lemery and Baglivi also tried this method of analysis, with no more definite results.

Thouvenal in 1778 was the first chemist to approximate the correct analysis when he was able to separate cantharides into four parts,-- namely, (1) a black matter which upon distillation gives a cold acid and a volatile salt, (2) a green oil having an acrid odor and vesicating properties, (3) a yellow substance which he considered as a simple wax and which is not worth considering in medical analysis, and (4) parenchyma of undetermined nature making up most of the weight of the insect. Beaupoil in 1803 made another analysis in which he found five substances present: a black extractive matter, soluble in water and insoluble in alcohol, a yellow matter soluble in both water and alcohol, an acid of unknown nature (possibly phosphoric), a green vesicating oil obtained only by use of ether or alcohol, and an insoluble parenchyma.

Brettoneau in 1810 was the first to isolate the vesicating substance, free from impurities. His method was to pulverize the insects, place in a test tube and pour on boiling ether. It was allowed to cool and evaporate on a glass plate, and a greasy layer was left. This layer was treated to remove impurities, leaving a white crystalline compound deposited in layers, insoluble in water and soluble in boiling alcohol. To determine its identity, he dissolved the crystals in an oil and applied it to the mucosa of the lips of a young dog, and in fifteen minutes a blister had formed.

Robiquet in 1813 also isolated the vesicant from cantharides, with ether as a solvent, and likewise used the physiological method to identify his product. He placed 1/100 part of a grain on a piece of paper and applied it to the edge of his lower lip; after some time pain and swelling resulted, and later his two lips were covered with blisters full of serous fluid.

Directly following Brettoneau and Robiquet, the compound which they isolated was analyzed by Regnault, given the formula $C_{10}H_6O_4$, and named cantharidin.

III. Place in Insect Body, and Biological Role.

After the compound cantharidin had been isolated and its properties worked out, there arose some controversy as to what part of the body of the insect contained it. Although, as we have seen, the ancient Greeks knew little of the nature of the substance, yet they did differentiate between different parts of the insect body, and were able to roughly grade the different organs in degrees of vesicating power.

Brettoneau used the simple method of macerating different parts of the body in chloroform separately. After three days it was evaporated, alcohol added and again evaporated, and crystals yielded. These crystals were tested out as before and vesication resulted in every case, so that Brettoneau concluded that cantharidin occurred in all parts of the body.

Neutwich in 1870 experimented on young adult beetles (unmated individuals) and found them inert; but his work was contradicted by Beauregard in 1885 who quoted the research carried on by Berthoud, Fumouze, Ferrer and Lissonde, 1859 to 1869. Their work may be summarized by the statement that the active principle is contained in the soft parts of all adult *Cantharis vesicatoria*, but not in the head, legs and elytra. Courbon, working with *Epicauta adspersa*, published similar results. Leidy (1869) using *Epicauta vittata*, found the vesicating principle in the blood and fatty matter peculiar to certain glands accessory to the generative organs and eggs. Then Beauregard experimented further on *Cantharis vesicatoria*, estimating the vesicating properties of different parts by applying to his arm moistened powders of the parts or a mixture of oil and crystals obtained by maceration in acetic ether, and evaporation. Following is a tabulation of his results:

Blood	positive
Muscles	negative
Malpighian tubes	negative
Hard parts with no blood	negative
Respiratory organs	negative
Digestive organs	negative
Male testicles	negative
Deferent canals	negative
Seminal vesicles	positive
~~Male~~ female genital apparatus (all parts)	positive
Eggs	positive
Larvae (just hatched)	positive
Young unmated forms (8-10mm)	positive

The question as to the biological role which cantharidin plays in the life of the insect has been very little considered. One paper was published in 1890 by Cuenot, in which he discusses somewhat fully the possible value of this substance to the beetle. The conclusion which he draws is that the fluid is protective in its nature, since the Meloidae are notable for their soft integument, and "were they not preserved by some other means they would be exposed to more destruction than the neighboring groups."

It seems, however, that one would be compelled to look further for the satisfactory answer to the question. First, because there are several other families of the Coleoptera which have less of a protective armor than the Meloidae, notably the Staphylinidae having practically the whole abdomen exposed, and the Lampyridae with even softer elytra; neither of these families have been proved to possess a fluid comparable to that of the blister beetles, although some Staphylinidae have been reported as poisonous. The second argument not satisfactorily answered by Cuenot is that these beetles are not protected from their natural enemies, such as birds, frogs and other insects, by this fluid, but may be eaten as well as any other insect, since the poison which they contain seems to be ineffectual against these forms. On the contrary, mammals are seriously affected, even fatally sometimes, but here again it has not proved a protection to the insect, but rather has had the opposite effect, since they have become an article of commerce by virtue of this very substance, and are hence more eagerly sought.

IV. Properties of Cantharidin.

The properties of this compound, cantharidin, found exclusively so far as is known at the present time in beetles of the family Meloidae, may be considered under two main heads: first, physico-chemical properties, and second, the physiological properties.

Briefly the physico-chemical properties as they are listed in the U. S. Dispensatory, 20th Edition, are as follows: cantharidin is the anhydride (lactone) of cantharidic acid, composed of colorless, glistening inodorous crystals. It is very slightly soluble in water, petroleum spirit and 90 per cent alcohol; more soluble in chloroform, acetic ether and acetone; soluble also in the fixed oils. Its melting point is 210-212°C; it volatilizes slowly at 100° C. It is soluble in solutions of NaOH, KOH, and NH_4OH, but is precipitated when acid is added. According to Ferrer, in a thesis read before l'Ecole de Pharmacie de Paris in 1859, cantharidin is also soluble in turpentine, hot concentrated sulphuric acid (the solution becomes colored and is precipitated by water) nitric, hydrochloric and acetic acids (all colorless solutions).

Witthaus in his "Toxicology" gives cantharidin the chemical formula of $C_{10}H_{12}O_4$, and describes it as a crystalline compound with four-sided rhombic prisms, soluble in alcohol, ether, water, chloroform and oils. Kobert's "Lehrbuch der Intoxikationen," (1906) states that the acid is a hydro-aromatic substance, and that it combines to form various salts, notably the calcium salt, whichhas some therapeutic value.

Physiologically, cantharidin has two distinct effects on the body, if one considers it only from the standpoint of higher mammals and particularly man. The physiological effect on lower animals has not been thoroughly worked out, and is an entirely different matter; thus it will have to be considered independently.

The first effect is blistering or vesication which results when pure cantharidin or any substance containing it is applied to the body surface in sufficient amount. A lesser amount causes rubefacience. Kobert gives the following details of the blistering reaction:

On the skin the poison leaves the well known blister, on which Kulisch has done some histological research. His drawing (reproduced by Kobert, fig. 50) shows a section through a blister on the human skin, very slightly enlarged. This section shows one large blister, though often there are many smaller ones. It contains besides fibrin, chromatin residuum and cell detritus, a great deal of clear serum; and strange to say, in this serum there are very few leukocytes, with an occasional eosinophile. On the contrary, there appears in the rest of the body, a striking leukocytosis which recently has very greatly reduced the therapeutic use of the Spanish fly.

Internally the effect of cantharidin or any substance containing it is severely toxic, and so far, no antidote for its effects has been discovered. Quoting from the 20th Edition of the U. S. Dispensatory, "it is a powerful irritant, with a peculiar direction for the urinary and genital organs. Genito-urinary irritation is ordinarily the first symptom produced by small doses of cantharidin, and if the dose has been large enough, it may amount to violent strangury, attended with excruciating pain and hematuria. Toxic doses of Spanish fly produce obstinate and painful priapism, vomiting, bloody stools, severe pains in the whole abdominal region, excessive salivation, fetid breath, hurried respiration, a hard and frequent pulse, burning thirst, exceeding difficulty of deglutition, sometimes a dread of liquids, frightful convulsions, tetanus, delirium and death. Orfila has known 24 grains to prove fatal. Dissection reveals inflammation and ulceration of the mucous coat of the intestine."

Probably the oldest source from which we get information concerning therapeutic uses without going back to the original Greek and Latin is in Moufet's "Theatre of Insects" from Topsel's "History of Beasts"(1658). Evidently a deep scholar, Moufet has compiled from the medical works of Pliny, Galen, Dioscorides, Hippocrates, Avicenna, Rhazes, Langius, Montegnana and others, a remarkably interesting discussion of the different diseases in which cantharides is supposed to be of value.

Direct quotations from this treatise will convey most clearly its quaint charm, as for instance, "their virtue and quality is to burn the body, to parch and to bring a hard scale or crustiness upon any part they shall be applied to; or as Dioscorides saith; to gnaw or eat into, to raise blisters, exulcerate or raise an inflammation, for which respect they mix them with such medicaments as are appointed to heal leprosies, any dangerous tetters and ringworms, or those that be cancerous." Cantharides are also used externally for removing corneous growths of all sorts, to take away "little hard red swellings," to prevent falling hair and in cases of "hydropsical persons." In the cure of gout the explanation of how it is effected is as follows: "I cannot here sufficiently enough commend their assured tried and approved use, being mixed with leaven, salt and gum ammoniacum, for the taking away of all goutish pains out of the hip (called the sciatica of popular sort) whilst they draw forth and consume from the center of the body (being there thoroughly and deeply impacted) to the surface, the matter of offending humours causing the griefs above said."

Pliny also recommends their use against the venom of a salamander, and as a diuretic.

Probably the most popular use in the Middle Ages was as an aphrodisiac or love potion, an instance of which is related by Moufet in a rather humorous fashion. "But here," he says, "I would counsel each one not to be knack hardy hold, in meddling with them, for these or the like intentions: for as they bring both health and help, being duly commixed and orderly tempered, not exceeding their dose and first quantity; so again if you fail in their due and skilful application or propination, they induce and drive men into most intolerable grievous symptoms and accidents, and otherwhiles to death itself. John Langius setteth down a true and very pleasant story, which in this place, because it maketh greatly for our matter in hand, I will not refuse briefly to describe it.

There was (saith he) at Bonony in Italy a certain rich and Noble young man of France, who falling extremely in love with a certain Maid in the same city, prevailed so far at length through his earnest importunities and incessant sollicitations, that at length they appointed a meeting place. So this lusty Gallant, being thus ensnared in the inextricable labyrinth of her beauteous Physiognomy, fearing deadly lest his heart should turn into Liver, or that he might faint and lose his courage before he should attain to his journey's end; in this his doubtful coaping ..... would needs know of a fellow-soldier and Countreyman of his, ... what best were to be done, to move him to a more vigorous courage; ..... lest he either should turn Craven like an overtired Jade, or else be utterly non suited, which was worst of all; who presently wished him to take some Cantharidies in his Broth, which the other at all adventures forthwith did.

And yet for all this, my restless gallant soon felt his whole body to be pockily torn, and miserably rent with sundry prickings and stingings, feeling moreover a strange taste in his mouth like the juice or liquor that issueth from the Cedar tree; stamping and staring, raging and faring like a furious mad frantic Bedlam, being almost besides himself through the extremity of his pain, virtiging and giddiness of his brain, with inclination to fainting or swounding: so being soft, troubled and perplexed, all sad, melancholike and mal-content, ... he bid his love adieu, full sore against his will you may be sure, and turning his back he trudged home to his lodging; whither being come, and finding no relief, but rather an increase of his torments, ... he lamentably besought and with weeping and tears most humbly craved and cried out for help, requiring the favor both of myself and another physician for the cure. ....

But after that my fiery Frenchman had recovered his former health, and that the unadvised Author of this rash counsel had very humbly entreated pardon at our hands for this his great fault, he protested solemnly with a great oath, that he would never hereafter prescribe any Physick to any man living. Thus saith John Langius in his first Book Epistola Medicinal, forty eight."

Another and "profitable" use which Moufet gives is "to beat Cantharides to powder, and convey a little of it into Apples, Pears, Plums, Figs, Peaches or Quinces: especially those that be ripest and fairest, and those that hang the lowest, finely closing it up again with a pill, which if any Thieves or Robbers of Orchards shall taste of, they fall within a while after into an intolerable sickness, whereby their theft is soon found out, and they are well rewarded with sour sauce for their sweet meat."

Berthoud (1859) states that "many believe that emanations from cantharidin are poisonous, sufficient to cause vertigo, fever, dysuria, hematuria, opthalmia and itching. There have been two cases reported (which have not been authenticated) where 16 grains of cantharidin wrapped in double paper carried in a pocket caused the above symptoms." Needless to say the symptoms observed were either grossly exaggerated, or due to some other cause, since such potency has never been reported by careful observers.

In this same thesis, Berthoud quotes several experiments on dogs from the "Traité de Toxicologie" of Orfila (1813). His conclusions are as follows:

1. A dose of 50, 60, or 70 cgm. introduced into the stomach of a dog causes death.

2. 384 grams of liquid received in distilling 250 grams cantharides with 1000 grams water killed a small dog in 14 hours.

3. 6 grams of oil of almond digested for 15 minutes 4 grams of powdered cantharides, injected into the jugular vein of a medium sized dog, killed it in 3½ hours.

It may be of interest to mention here that Orfila attributes the death of his subjects to mechanical irritation. In the first experiment, this might be a logical conclusion, but in numbers two and three, where an aqueous and and oil extract were used, there seems to be little if any basis for this decision.

In the same year that Berthoud's thesis appeared, 1859, Ferrer's thesis, mentioned above, included the statements from several toxicologists. Briefly, not all animals are affected similarly: Pallas has found hedgehogs immune from the poison; Bouchardt experimented on such cold blooded animals as crayfish, fish and frogs, and found them not affected nearly so much as warm blooded animals. For man and the animals which most resemble him, cantharidin is a violent poison.

In 1860, M. Vezien, a medécin militaire in the French Expeditionary forces reports an extremely interesting case of canthariasis under the title, "Note sur la cystite cantharidienne causeé par l'ingestion de grenouilles que so sont nourries de coleoptéres vesicants."

The military doctors serving in Algeria often reported a genito-urinary inflammation caused by eating frogs. The diagnosis of urethritis invariably made was wrong, as Vezien proved at the time of a rather severe epidemic in May and June, 1849, (not published till 1860) in the province of Constantine. At this time certain vesicating insects (Mylabris vicina) appeared in great numbers along the banks of the brooks, and were fed upon by frogs (as they ascertained by dissection). The special irritating fluid of these Coleoptera then passes into the flesh of the frogs.

A certain number of men who ate the flesh of these frogs then developed a typical cantharidin poisoning. The element of individual variation enters in here, since some of the men who had eaten the frogs were not sick at all, some were very slightly affected, while the majority were rather violently ill.

The only control found necessary was to abstain from eating the frogs, and complete recovery follows in two to three days. It is worthy of note that a small frog devours daily enough of this poison to kill an animal as large as a man, and yet suffers no ill effect whatever.

It appears that Leptopalpus chevrolati, another Meloid which is often found in the same situations in Northern Africa is also capable of causing the same sickness. This observation does not seem to be as well substantiated as the first one, however, and must, perhaps, be disregarded.

In 1893 another French military physician, Meynier, published in the "Archives de Medecin et de Pharmacie Militaires" an article of similar content. After a rather exhaustive account of the position and route of the military unit with which he was allied, and a full description of all symptoms (which tally exactly with other accounts of cantharidin poisoning) he deduces that the frogs eaten by the soldiers must be the vehicle of an organic poison.

Inspection of the localities where the frogs were found revealed large numbers of Mylabrides which they had fed on very extensively, and which had undoubtedly poisoned the flesh. Control of the epidemic was as before, through denying access to the river banks, so that no frogs could be eaten.

Further knowledge of the physiological action of cantharidin was found in a thesis written by Lissonde in 1869 which had for its title "De la Cantharidine." The thesis was divided into eight parts, one of which concerned the action of cantharidin on economic animals.

0.05 centigrams of cantharidin are as active as about 8 grams of the powdered insects. The substance acts better in a solution of chloroform or any oil or grease. A rabbit and a cat are killed by 0.025 milligrams of the principle, while it requires 0.25 milligrams to kill a large dog. The animals thus poisoned succumb not because of organic lesions but because of a general action on the vital functions.

Experiment 1. A young cat swallowed 0.025 milligrams of crystallized cantharidin. In fifteen minutes it showed a vague disquietude, a stupor gradually increasing, trembling limbs, partial paralysis. It falls down repeatedly, has bloody saliva, anuria, and dies in a convulsion in four hours.

Experiment 2. 0.014 milligrams crystallized cantharidin dissolved in chloroform was absorbed by a rabbit in a sub-cutaneous injection. It died five hours afterward, and presented the same symptoms of poisoning. Autopsy showed only a general systemic inflammation. Sectioning kidneys showed a little congestion, but no lesions of the renal epithelium. There were ulcerations and lesions in the stomach caused by direct contact with cantharidin.

In 1890 the most extensive single work on vesicating insects was published by H. Beauregard in Paris. "Les Insects Vesicants" is a volume of several hundred pages which is divided into four parts; the first deals with anatomy of the Meloidae, taking up the integument, skeletal system, digestive apparatus, circulatory, respiratory, nervous and reproductive systems in the order given.

The second part concerns the physiology and pharmacology of the beetles, and is the section which was of greatest value in the preparation of this paper, since it is the phenomenon of vesication which first suggested the experiments to be carried out. Beauregard first considered in some detail the digestive phenomena, both from the mechanical and chemical standpoints. In the second chapter, which is headed "The seat of the active principle" there is included a list of all vesicating species, giving the experimenter, date, and conditions under which each species was analyzed, in chronological order. It is not necessary to go into this list in detail here, since it will be considered more fully later.

There follows a resume of the methods used to extract cantharidin from several species of vesicants, qualitative and quantitative analyses of the species most commonly employed in medical practice, as well as analyses of several species supposedly vesicating. To this resume of other authors' work, Beauregard adds then his own conclusions on Meloidae and beetles of other families, enlarging appreciably the catalogue of vesicants. Therapeutic uses, collection and preservation of specimens occupy the remainder of the second part.

The third part is an account of geographic distribution and life histories, including development and larval anatomy of many of the genera. The last section deals wholly with classification, and is followed by a bibliography of 115 citations.

In the section dealing with the therapeutics of cantharidin some interesting new facts are brought to light. Quoting from Galippe in his "Etude toxicologique," cantharidin compounds were once extensively used as aphrodisiacs or love potions, and sold under names such as "Diabolini de Naples, Beaumes de Gilead, Pastilles aromatiques." Many of these preparations were secret formulae and recommended clandestinely, as in the incident quoted from Moufet in 1658. At the present time such use of these preparations has been almost completely abandoned, and there remains only the tincture and wine of cantharidin as internal remedies. In cases of gonorrhea, dropsy and rabies, the Greeks use Mylabris bimaculata internally. By far the most important therapeutic value of cantharidin is in its preparations for vesicating, such as the cerate, collodion and epispastic liquor.

Some added facts of interest are given in Kobert's "Lehrbuch der Intoxicationen" (1906, Vol. II), who says that people whose occupation demands that they work with cantharides, druggists for example, are liable to be poisoned if proper precautions are not taken that the powder is not inhaled. An interesting application of this may be mentioned here; namely, that men in Germany were sometimes found guilty of having inhaled Cantharides to simulate illness in order to escape military service, and the symptoms resulting were those of a diphtheritic infection.

V. Catalogue of Vesicants.

The ancients had some knowledge of the difference in vesicating power between various kinds of blister beetles, as the preceding notes have shown. Yet their knowledge was limited to a very few specimens, and these few somewhat vaguely defined as the blister beetles with "the yellow banded fat body" and "those all of one color," etc. It is true that there was much discussion of the part of the insect which was most active, but probably because of the unsatisfactory state of systematic literature up to the time of Linnaeus, Fabricius and others, little attempt was made to gather together a list of all vesicating species.

Following the isolation of cantharidin in 1810-1813, Bretton-eau applied the same solvents to various other insects with the object of determining as many of the blistering species of the Meloidae as possible. In this way the activity of the genera _Meloe_, _Mylabris_ and _Cerocoma_ was established, but he found _Sitaris_ to be non-vesicating. In 1829 Farines, working on the Meloidae, recounted the activity of some of the species of the genus Zonitis; Z. praeusta was active and Z. quadripunctata inert.

In 1835, the pharmacist Leclère, using Brettoneau's method proved the vesicating power of nine genera, namely Cantharis, Cerocoma, Dices, Decatoma, Lydus, Oenas, Meloe, Mylabris and Tetraonyx. He found Zonitis, Sitaris and Nemognatha inert, and did not experiment on Gnathium. In the genus Mylabris, Leclére found M. pustulata entirely inert, while Dr. Collas 1853, published a report to the effect that M. pustulata and M. punctem were both active, the former even slightly more so than Cantharis vesicatoria.

A memoir by Lavine and Sobrero in 1845 gave positive results for seven species of Meloe, which need not all be cited here. Courbon (1855) published his observations on "les Coleopteres vesicants des environs de Montivideo" and recognized vesicating properties in the genera of Lytta and Epicauta. The list of active vesicants is thus gradually increased, and Ferrer in a thesis read before l'Ecole Superieure de Pharmacie de Paris reviewed all the work done previously, adding to it his research on several species. The new genera which he declared positive are Lydus and Hycleus, and he also added to the list a number of species in genera already established in this catalogue.

Beguin,(1874) tried out a large number of species of Meloe and Mylabris, and from his results concludes that these entire genera are vesicants. He also found Sitaris, Zonitis and Lagorina active, but did not experiment on the genera Stenoria, Nemognatha, Horia, Gnathium and others.

Beauregard in his work cited previously, verified the vesicating power of all the aforementioned genera and established the

4-21 6M

activity of Coryna, Alosymus, Cabalia, Nemognatha, Henous and Stenoria. He tried out so many species, and his results were so uniformly positive save in the case of two genera, that he felt justified in stating that all Meloidae save the group Horiides (genera Horia and Tricrania) are vesicating. Kobert,(1906) included in his list a great many species of Meloidae belonging to genera mentioned by previous workers.

In 1917 Chalmers and King published a very interesting paper concerning blister beetles as a public nuisance in Khartoum. It was found that in the morning and evening the beetles were attracted into the houses by the lights, and in some instances they proved annoying to people eating their evening meal in the garden. Natives do not suffer at all, but white people seem to be excessively bothered. The beetles do no harm just by crawling on the skin, but if they are annoyed by clothing or rubbed in any way a blistering fluid is ejected which causes a round blister, or if the insect is moving, the fluid makes a line aross the skin which subsequently blisters. In twelve to twenty-four hours itching occurs, followed by blisters full of yellow serum in a reddened area. The beetles causing this epidemic are Epicauta sapphirinia, E. tomentosa and Mylabris nubica. An epidemic of this type of dermatitis was also reported from Senegal, caused by Epicauta flavicornis, Cantharis vestita and C. melanocephala.

The references to vesication caused by insects other than Meloidae are almost endless in number, but few, unfortunately, show beyond doubt that a true vesication is established. Brettoneau and Leclére already cited, have experimented on various Coleoptera outside the family Meloidae, but found only negative forms.

In 1827 Mitouart and Bonastre published a note on the vesicating power of Calendra granaria, brought to their notice by Peneau, which will be discussed more fully under the work on that beetle.

Beguin and Lallemand have taken 100 beetles at random from among all genera, and have found that none cause true vesication, although some, such as Coccinelids, Carabids, and Chrysomelids, when applied on the skin, may result in slight inflammation or even an eruption.

An interesting series of cases was reported by Tissérant in 1886, under the title "Accidents provoques chez le cheval par Blaps mortisaga." This man was a veterinary surgeon, and relates that from time to time horses were brought to him for treatment, which exhibited no symptoms save that they could not eat, and showed a general swelling of the epidermis in spots on the nose, lips and cheeks. Sometimes the mucosa around the mouth and the nasal fossa also showed erosions. Since the disease usually occurred in horses which had been employed around houses in the process of construction, the diagnosis given was that of burning by lime which had been snuffed up.

There was reason to doubt this diagnosis, however, when two horses were brought in at different times by the same man; each horse showing the same symptoms after spending the night in the same stable. Since these symptoms of a peeling, blistered face, mouth and lips burned and swollen and abundant saliva, had not been present before they were in the stable, the conclusion forced upon the doctor was that some peculiar situation in the stall or manger caused the illness. A thorough search in the daytime yielded no results, but observation late at night revealed the fact that the manger of the affected horses was full of large black beetles.

Tissérant did not hesitate to lay the blame on these insects, which he identified as Tenebrionidae (Blaps mortisaga), since observation showed him that they possessed the power of ejecting from the anal opening a very abundant acrid liquid when they were annoyed. He says, "This liquid is not only acrid, but can also produce violent vesication on delicate animal tissues." This author does not attempt a qualitative study of the fluid, but contents himself with theorizing about it, to the effect that "if these insects have the power to eject from the anus a special liquid it is reasonable to suppose that it is not so acrid or vesicating during its whole adult life (which seems to last several months) but only during the rather limited time of the breeding or mating season."

Goossens (1886) reports on some caterpillars, Cabalia segetum, which leave in the nest the hairs and spines of their shed larval skins. This nest, soaked in 90 per cent alcohol for several days, and the filtrate evaporated, leaves a deep green oily substance. This oil applied on oiled silk to the skin causes a blister in ten hours, a result which is very similar to those achieved by Fabre some years later, and which will be discussed presently.

A note on an insect belonging to the order Hemiptera, and which possesses vesicating properties is published by Arnaud and Brongniart(1888). This is a Cicada found in China, and determined as Huechys sanguinolenta. Research on this insect shows that it causes a vesication less decided than that from true cantharides, but still a slight raising of the epithelium and redness, and a small amount of fluid in the vesicles. Chemical experiments carried out to extract the supposed cantharidin from the insects disclosed only a vesicating green oil containing no crystals. The conclusion given was that the vesication resulted from an unknown substance in the oil, or from the oil itself, as in the case of croton oil.

In the same year Fumouze analyzed Huechys sanguinea, with the two main objects of distinguishing the source of the coloring matter in the abdomen and the peculiar odor which they emanate. Merely as a side issue the substances isolated were tested to determine their vesicating power, and Fumouze found them all entirely inert. This difference in results might possibly be explained by a difference in methods, or in the original material; but evidently there is a need of further research on the subject.

A note similar to that of Tissérant was published by Bassi (1893) entitled "Eczema vesicoloso labiale e nasale del cavallo causato in maniera indiretta par Blaps nero." There is some doubt as to whether the two species, Blaps nero and Blaps mortisaga, are not identical, but in any case the manner in which these Tenebrionids affect the horses is exactly similar.

Kobert includes in his discussion of irritating organic substances under the head of insects a list of insects which are supposedly vesicating, but neglects to give any of the circumstances surrounding the discovery. Therefore, without adequate data, the list must be regarded as purely tentative. Beside the Meloidae of known vesicating power the list includes: Chrysomelidae, Leptinotarsa decemlineata, Chrysomela popula, C. tremulae, Megalopus sp., Galeruca sp., Timarcha sp., Lina tremulae; Coccinelidae, Coccinella septem-punctata, Anatis sp., Chilocorus sp., Adalia sp., and

Eriopis connexa; Tenebrionidae, Blaps mortisaga; Cucujidae, Silvanus surinamensis; Staphylinidae, Megalopus sp., Paederus cerebripunctata, P. columbinus, P. peregrinus, P. flavicornis, P. tomentosa; Gyrinidae, Gyrinus natator; Scarabaeidae, Cetonia aurata, C. floricola; Carabidae, Brachinus crepitans, B. explodens; Carabus niger, C. auratus; Dytiscidae, Dytiscus marginata; and Aenas afer, a Spanish form substituted for Cantharis vesicatoria. This list simply emphasizes the need of adequate research on the subject of insect poisons, rather than serving as report on completed work.

Da Silva (1912) reports on vesication caused by a Staphylinid, Paederus columbinus, which at certain seasons is exceedingly numerous along the banks of rivers in the interior of Bahia in South America. The laborers cultivating the land there are severly affected by coming in contact with these beetles, which liberate on the skin a caustic and blistering fluid. The first symptom is inflammation, followed by intense pruritis and finally ulceration. Sometimes these ulcers are numerous and extended, are very stubborn to treatment and leave deep scars. They occur usually on exposed parts, and attain sometimes a very large size. No research has been done on the active principle involved.

A similar report is made by Eysell (1913) for another Staphylinid, Paederus perigrinus, which is found in the Island of Sunda in the Malayan Archipelago. This beetle causes erythema and vesication, followed by eschar formation. Rodhain and Houssiau (1915) cite an epidemic of seasonal vesicular dermatitis which occurred during April and May in Leopoldville. This dermatitis is provoked by another species of Paederus which is not further specified. The clinical picture produced by application of the irritating liquid of this beetle is as follows: "Blisters either in round blotches or long lines, surrounded by an erythematous zone; the blisters are filled with a seropurulent yellowish liquid. The initial redness is followed in twenty-four to forty-eight hours by this blister, which persists for four or five days, and is terminated by desquamation of the epidermis, leaving a slightly pigmented area which fades gradually."

The etiology of this dermatitis was accurately worked out by experimentally rubbing the beetles on the skin, thus proving the possession of a substance similar to cantharidin.

Ross (1916) reports a similar dermatitis in Nairobi in British East Africa, which is caused by a Staphylinid of this same genus, but different species, Paederus cerebripunctata. When the beetles walk across the skin and are annoyed in any way an irritating secretion is left on the skin which causes an inflamed vesicular streak to appear, and subsequently a dermatitis exactly similar to that described by Rodhain and Houssiau. Here also the etiology was worked out and not left to chance observation, so there seems to be little doubt that some Staphylinidae are actually vesicating.

The work previously referred to by Fabre was written at various times and published separately, but gathered together and the English translation published in 1916. This includes a list of experiments on various Lepidopterous larvae, particularly the Pine Processionary and the Arbutus caterpillar (Liparis sp.). In both of these instances the nest containing the cast skins and hairs of the larvae need merely to be touched to cause an intensely itching eruption. These nests soaked in alcohol or ether, as in Goossens, yield a fluid which causes acute itching, burning and finally pustules after being bandaged on for some time.

Yet Fabre got the same result when he tested the excrement of various larvae treated with ether. The blood of the caterpillar also yields vesication, so Fabre decides that an urticating excretion is common to all caterpillars, and is not a special mode of defense in a few forms. The fluid ejected when any mature insect emerges from its pupa is also capable of bringing about the same result, even insects of the order Coleoptera. Those insects which have incomplete metamorphoses possess an urticating fluid in their excrement; so it occurred to Fabre that possibly, since all insect excrement possessed this property, it might be found in that of other animals as well. But after trying out excrement of birds and a few other animals and achieving no results, he concludes that the active principle is not uric acid, but a special fluid peculiar to insects.

It may be mentioned here that the writer, among others, has repeated some of Fabre's experiments on miscellaneous Coleoptera and Lepidoptera, but always with negative results. Therefore the conclusion is reached that Fabre's statement concerning the possession of an urticating fluid in all insects is somewhat too broad, and his experiments might be the result of an individual sensitivity resulting from his previous work on the Pine Processionary and others.

VI. Methods of Extraction.

Since Meloid beetles have been used in medicine for so many centuries it is to be expected that there have been a great many methods devised for extracting the active principle from the body of the insect; but owing to the fact that this paper is not written from the standpoint of pharmacy, the writer will attempt only the briefest summary of some of the more important ones. In the very early works, unfortunately, there is no specific mention made of what process was used. We find simply the word "cantharides" to designate the substance. This might mean, however, that the powdered insects were often used without employing any solvent or method of extraction; a view which is borne out by the notes in Moufet (1658).

Here we find that cantharides are mixed with "such medicaments" as are appointed to heal various diseases, or more specifically, with plasters, cerates, tar or lime for external use. Internally cantharides are administered mixed with sheep or goat suet, or with other diuretics. For gout a preparation of cantharides in leaven, salt and gum ammoniacum is recommended, or as a love potion it is mixed with any beverage like tea or broth.

Moufet stresses the fact that the safest way to adminiter cantharides is whole, using all and every part, since authorities are so much in dispute concerning where the substance is located in the body of the insect.

In the notes previously given in the section under "History of the discovery of cantharidin" the methods used by the later workers were mentioned, such as treatment with boiling water followed by boiling alcohol and ether, employed by Robiquet in 1810, and by Brettoneau a few years later. Carpenter (1831) used a new method and devised a preparation called Oil of Cantharidin which is as follows: "The cantharides are boiled in sulphuric ether, which takes up with the cantharidin a greenish colored oil sometimes combined with fatty matter. This may be separated from the cantharidin by washing the crystals in cold ether, which is however, unnecessary, since when it is thus combined it exercises its epispastic effect equally well. Cantharidin, when thus washed shows beautifully prismatic crystals, entirely colorless. Combined with an oil it communicates to the latter, in a high degree, its vesicating properties. It is well to dissolve the crystals in strong sulphuric ether, and mix the ether and oil together, which will make a clear solution. They are with difficulty soluble in oil alone; the sulphuric ether is also an advantage by its evaporating on the part where it is applied, thus leaving the oil more circumscribed. I have tried the oil repeatedly on my own person, and found it invariably to produce a blister in about the same time as the ordinary ointment, and it is so mild that it generally produces very little sensation." A little later Wolff also employed ethereal extraction.

Warner,(1856) evolved a rather complicated method, but one that is accurate, and successful in quantitative analyses. Working on C. vesicatoria, the powdered insects are first heated in a retort with water, until the distillate is opalescent. The distillate is then agitated with ether and allowed to evaporate. Allowing the residue to remain in the retort, boil in successive portions of water till all the soluble substance is extracted. The vesicating property then, is all contained in the filtrate. The decoctions obtained are mixed and evaporated below 212° F. to nearly solid. Add alcohol, boil and strain. The residue is then precipitated by subacetate of lead, and the rest evaporated to yield yellow matter of cantharidin. Dried by heat, mixed with one-half its weight of pure dry animal charcoal, moistened with ether, it is allowed to stand covered for twenty-four hours. Percolate with 2 oz. sulphuric ether, evaporate, and pale yellow crystals of cantharidin remain.

After this method, C. vesicatoria yielded 2.03 gr. per 100, C. vittata yielded 1.99 and Mylabris cichorii yielded 2.13 gr.

In 1864 Morteux made known a process based on the insolubility of cantharidin in carbon disulphide. The extract obtained with chloroform was treated with CS_2, which dissolved out the impurities, and left the cantharidin in the chloroform nearly pure. Then recrystallize by the aid of chloroform and boiling alcohol. A little later Dragendorff (1867) and Beguin (1874) showed that CS_2 does dissolve a trace of cantharidin.

Another method published by Galippe (1876) made use of acetic ether, and was most efficient in that it dissolved more cantharidin than any other fluid. He ascertained that at 18° C. acetic ether extracted 1.26 gr. cantharidin from 100 gr., while chloroform at 18° C. extracted only 1.20 gr. This liquid evaporated gives beautiful crystals in the form of oblique prisms, rhombic at the base.

Fahnestock (1879) contributed several processes which were not radically different from those used before, but which improved the results in that more cantharidin is extracted and is left in purer state. One method is to macerate with chloroform, filter, distill off the chloroform, pour the residue in a dish and wash out the retort with more chloroform, adding it to that in the dish. Allow it to evaporate to a thick extract and treat it with CS_2. This leaves the cantharidin impure, and it is then dissolved in alcohol and allowed to evaporate, leaving pure crystals. A second method elaborated by Fahnestock is to exhaust the cantharides with acetic ether by displacement, then distill off most of it and allow the rest to evaporate. The residue is treated with carbon disulphide, dissolved in hot alcohol and cooled. Crystals of cantharidin then separate out.

A still more complicated process is to digest the cantharides in KOH, neutralize with hydrochloric acid, dry and treat with petroleum benzine by displacement. It is next evaporated, exhausted with chloroform and treated as in the first method cited. This yields slightly less pure cantharidin than the first method, or 2.5 gr. per 100 gr. compared to 2.8 gr. per 100 gr.

A simple method proposed by Goossens (1881) is to macerate the cantharides in chloroform, and then add it to tepid water. Greasy droplets containing cantharidin separate out, but it is not possible to isolate pure crystals by this method. Girard, following Goossens, used this process but was also unable to reduce the "greasy droplets" any further.

Two pharmacists, Greenish and Wilson, in 1898 made a rather exhaustive study of the pharmacy of cantharides and published a paper containing processes for removing free and combined cantharidin, assays of various preparations, and formulae for several of the more important preparations included in the United States pharmacopeia. Their process to determine the total amount of cantharidin is as follows: Take 20 grams of the cantharides and mix in a mortar with 25 cc. of a mixture composed of 1 volume glacial acetic acid, 2 volumes rectified spirit and 3 volumes of chloroform. The moistened mass should be covered for one hour and then allowed to dry spontaneously. It is then packed in an extractor and exhausted with pure chloroform for one hour, and the chloroform solution next placed in a separator with water and the acetic acid remaining from the first step neutralized with KOH and shaken well. The chloroform layer is poured off and evaporated cautiously. The residue consists of fat containing crystals of cantharidin; the fat is removed by washing with petroleum spirit (the washings should be saved) and there is left in the dish crystals of cantharidin mixed with green resinous material. After the resin has dried it is washed with absolute alcohol until the green color disappears, and pure colorless crystals of cantharidin remain. The washings contain some cantharidin which can be recovered by special methods.

The formulae for a few of the preparations of cantharidin may well be included here. One of the most commonly used is a plaster of cantharides. It may be made by taking 1 part cantharidin dissolved in chloroform and added to 999 parts of a mixture composed of equal parts yellow wax, prepared suet and resin. The chloroform solution is added to the other ingredients which have previously been melted together on a water bath, and heated until the chloroform has passed off. This mixture should be stirred until cold and then applied as a plaster; a blister results in five to six hours.

A "liquor epispasticus" may be prepared by taking cantharidin 1 part, castor oil 6 parts, resin 3 parts, and enough acetic ether to make 300 parts. The cantharidin is dissolved in the acetic ether and the resin and oil added slowly. Vesicating collodion has the epispastic liquor as a base-- 40 fluid parts to 1 part of pyroxylin. The pyroxylin is dissolved, and application of the resulting compound raises a blister in approximately eight hours.

A plaster (emplastrum califaciens) which is supposed only to cause rubifacience without blistering, contains 1 part cantharidin dissolved in chloroform, added to 199 parts of olive oil. This mixture is added to 4800 parts of previously melted resin plaster and heated till the chloroform is evaporated and then stirred till cold.

Cantharidin vinegar is prepared by adding 1 part cantharidin to 200 fluid parts of glacial acetic acid. This is added to enough acetic acid to make 2000 fluid parts and all dissolved on a water bath. A tincture of cantharides for internal use is made of 1 part cantharidin dissolved in 100 fluid parts of chloroform and added to enough "rectified spirit" to make 10,000 fluid parts.

These different methods of extraction serve but to emphasize the true and natural solvents of cantharidin, whether in the free or combined state. Alcohol, chloroform, ether, acetic ether and the essential oils are most efficient and give results which are qualitative, although not in any sense accurate enough for a quantitative study. But since the object of this work was merely a qualitative study of several suspected beetles, the simplest methods are all that need be applied.

Several of these solvents, namely ether, chloroform and acetic ether, were tried out tentatively with Mylabris in order to ascertain their effect on the skin. All three preparations proved to be vesicating, so that it was determined to use these, with several others, in checking the experiments on the vesicating effects of the various Coleoptera studied, as will be shown in the following section on Calendra granaria.

VII. Calendra granaria.

In 1827 Mitouart and Bonastre reported on some work done by Peneau, a pharmacist at Bourges, before the Pharmaceutical Section, Academie Royale de Medecin. M. Peneau observes that besides causing incalculable injury to wheat, the weevil, Calendra granaria, is thought to be the cause of colic produced by eating bread made from flour of infested wheat. After crushing the fresh insects in sweet almond oil and applying them on the skin, Peneau claimed that in five hours a "lively irritation" resulted, somewhat less strong than that produced by a plaster of cantharides. (He also mentioned the presence of gallic acid in the weevils, which is of no importance in this connection.)

The commissioners of the society repeated Peneau's experiments and failed to note any appreciable irritation on the skin from such an application. They also tried distillation with water, and obtained only a pale and inert liquid; this aqueous extract, treated with alcohol and ether, has a styptic or astringent taste. This property is very well marked, but nevertheless not strong enough to cause irritation of the tongue, lips or pharynx. Some months later Henry and Bonastre again did some analytical experiments on "les charancons" to endeavor if possible to isolate some principle analogous to that in the cantharides, which Peneau seems to have indicated. Their most careful analysis resulted in separating out eleven substances, not one of which bears any relation to cantharidin.

To quote: "In order to once more assure ourselves if the weevils were vesicating, we made a powder of them in the fresh state, and formed two blister plasters with the paste. These two plasters were applied on two subjects, one robust, vigorous and middle aged, the other very young and more delicate; they have produced neither burns nor rubifacience in the space of 24 hours. We have thus confirmed the experiments of our colleagues."

In strong contrast to this view we find a note by Mills in 1835 on these weevils, in which he describes the mode of entry of the adult beetle into a grain of corn or wheat. He then goes on to say: "An old medical gentleman in Madeira assured me that he considers the wings and crustaceous parts of the weevil so heating to the system as to be almost as injurious as Cantharides, taken internally, on a slow scale."

This latter view is also brought out by Walsh and Riley (1869) in an article entitled "Poisonous Flour." They quote Dr. W. D. Hartman of West Chester, Pennsylvania, who said that "Sitophilus granarius (Syn. Calendra granaria) in the South had been used successfully as a substitute for Spanish fly. The added advantage is that Sitophilus granarius does not cause strangury." Information is lacking as tohow the discovery of its use was made, but it probably was necessary because of the difficulty of getting real Cantharides during the war. (American Civil War).

Walsh and Riley go on to say that "grain weevils often are ground up into flour, and while the elytra, snouts and legs are sifted out, the body probably is pulverized into flour, thus making the flour poisonous. Weevils have the same medicinal properties as Spanish flies and would therefore constitute a violent and dangerous internal drug." Thus it seems probable that Walsh and Riley have accepted in its entirety the views of the unknown "medical gentleman of Madeira" and of Dr. Hartman in stating that "weevils have the same medicinal value as Spanish flies," since there is no record of their having tested out this property, or analyzed the beetles in any way.

The usual psychological effect of believing without investigation a statement observed in print, seems to have occurred in this case, since we find in Herrick's "Insects Injurious to the Household" (1914) what is probably a direct quotation from Walsh and Riley. He says, "A curious, interesting, and perhaps important bit of knowledge concerning this insect (C. granaria) is the fact that it has been used successfully as a substitute for Spanish blister beetle, with this added advantage, that it does not produce strangury. It was apparently used for this purpose in the South, perhaps during the war, when the real Spanish beetles were not obtainable. So far as is generally known, however, the granary weevils are not used for this purpose at present.

omit [In Kobert's "Lehrbuch," cited above, there is included in a summary of all poisonous beetles, the names of Calendra granaria and its closely related species Calendra oryzae.]

The only other mention which the writer has been able to find of a weevil possessing medicinal qualities occurred in Blanchard's Zoologie Medicale, Vol. 2. Larinus nidificans, found in Asia Minor and Syria, builds on the stem of its host plant a shell or case which is used to make an infusion of reputed value in the treatment of respiratory infections. The related form, Larinus maculatus, found in Persia yields schakar il-ma-ascher or "sugar of nests," which is used for this same purpose.

With the foregoing literature as a foundation, experimental work on Calendra granaria was undertaken. These experiments were of three types: (1) feeding experiments, in which rats, mice, frogs, chickens, a rabbit and a cat were used; (2) vesicating experiments, which were carried out using the writer as subject in all but a very small number of trials; (3) subcutaneous inoculations, using rats and chickens as subjects.

Before taking up the discussion of the feeding experiments an outline of the preparation of material is as follows: The weevils were collected from a culture in barley very kindly furnished by Dr. R. N. Chapman of this University. In all cases they were killed by heat to obviate the possibility of any other poison used as an insecticide entering in.

The insects were then dried slowly in an open dish in an oven at approximately 60° C. and used whole, or were ground to a coarse powder. The powder was fed dry in some cases, or in gelatine capsules, but usually was mixed with distilled water in sufficient amount to make a sort of coarse paste to facilitate feeding. More rarely the ground insects were suspended in water and administered with a pipette, or were extracted in water and the clear solution used. Three trials were made with an alcoholic extract prepared by macerating the ground insects in acid alcohol and then diluting it; but there was a question here of the toxic qualities of the alcohol itself, so that this solution was immediately abandoned.

A check was run on all feeding experiments, using commercial "Spanish fly" or blister beetles prepared from various species of Mylabris. This is put out by a wholesale drug concern under the name of "Blistering flies" or Chinese blistering beetles.

A. FEEDING EXPERIMENTS.

Experiment I.

1. Adult white mouse fed 20 beetles dry, on Jan. 29th. No ill effects were observed Jan. 30th or 31st. On Feb. 1st it received twenty more beetles, making a total of 40; no ill effects observed then or at any time later.

2. Adult white mouse fed 15 dried beetles on Jan. 29th, and showing no effects it was given 20 more on Feb. 1st, total 35. No ill effects observed.

3. Adult white mouse fed five beetles Jan. 29th, and 20 beetles Feb. 1st, total 25. On Feb. 2nd it was dead, its symptoms unobserved. Dissection showed no unusual inflammation or other gross pathological condition which might have caused its death. It seems unlikely, however, that it was poisoned by the beetles, in view of the larger numbers which other individuals consumed.

4. Adult white mouse fed 40 beetles on Feb. 12th, and the next day it received 60 more, totaling 100. No ill effects were observed during four succeeding days, and at the end of that time it was killed.

Check on Experiment I.

1. Two adult white mice fed approximately 0.5 gram <u>Mylabris</u> at 9:55 A.M. Almost immediately convulsions began, which increased in severity until the mice were unable to stand, but were lying on their sides, with convulsive tremors at intervals of two seconds. This continued till 11:00, when the mice seemed exhausted, with gasping for breath becoming weaker and weaker. At 11:30 the mice

were both nearly dead and were chloroformed and dissected.

3. Coincident with this a mouse from the same cage, but which had not been fed any insects, was killed and dissected for comparison. The normal mouse had the characteristic yellowish pink color of body wall and viscera, contrasting strongly with that of the poisoned mice. The latter showed a distinctly darker and congested appearance, and upon opening the alimentary canal it was seen that there was intense inflammation as far as the middle of the small intestine (or as far as the powdered insects had progressed). The stomach was distended somewhat with gas, and showed traces of the blackish powder. The lungs were also very much congested, in contrast to those of the normal mouse. The animals had not lived long enough after feeding to show any effect on the excretory or reproductive systems.

Experiment II.

1. The mouse from the preceding experiment, number 1,4, was fed to a young cat, which was not in very good condition. (The reason was discovered later.) The cat showed no ill effects after the feeding.

2. On Feb. 19th this cat was given 200 Calendra in a capsule, following which it developed a very severe diarrhea which lasted four days. During the entire period of the experiment the cat was allowed the freedom of the laboratory where it might easily have picked up something that would cause the intestinal disturbance. Subsequent dissection showed also that the cat was suffering from coccidiosis, so that it seems necessary to exclude this experiment from consideration.

Experiment III.

1. A young rabbit in good condition was fed 100 _Calendra_ in a capsule, and observed closely for one week. During this time the only symptoms seemed to be restlessness and lack of appetite, which might easily be due to its close confinement while under observation. At the end of the week the subject was fed 350 more beetles, making a total of 450, but it showed no ill effects whatever.

Since the effect of cantharides on rabbits, dogs and cats are clearly outlined by Berthoud (1859) and Lissonde (1869) as quoted above, it was not thought necessary to perform check experiments for numbers II and III, but rather to accept their results.

Experiment IV.

1. Three young white rats in good condition were each forced to swallow a capsule containing 260 grams of powdered _Calendra_. Since close observation showed no unnatural reaction at any time after the feeding, the rats were not dissected.

Check on Experiment IV.

1. A rat from the same litter and kept in the same cage as those in the foregoing experiment was fed at 9:30 A.M. powdered _Mylabris_ moistened in water to a thick suspension to facilitate feeding. At periods extending over two hours it ingested approximately 250 grams, and immediately began to show malaise. There were no strong convulsions similar to those of the mouse, but only convulsive shivering. It crouched down in a corner of the cage, making the peculiar noise which indicates in rats a state of ill health. The condition remained precisely the same until 4:30 P.M.

when it was last observed. The next morning at 8:30 the rat was dead, and had apparently been dead for some time. Dissection showed, in comparison with a normal rat which was killed by chloroform, that the entire body cavity was deeply inflamed, the lungs haemorrhagic, the stomach enormously distended with gas, although not highly inflamed. The small intestine was inflamed throughout its length as far as the caecum; the large intestine was normal, and dissection showed that the powder had not passed that far; the kidneys and reproductive organs seemed normal.

Experiment V.

1. Six specimens of *Rana pipiens*, all about half grown, were each fed 25 *Calendra* on Feb. 7th. They were not poisoned, and two days later they were again each fed 25 beetles. Two weeks later one died, but probably from lack of food, since they were not fed during the experiment, and this one was apparently smaller and weaker than the others. Feb. 21st all but one were killed to look for intestinal parasites, having shown no ill effects whatever from the feeding. This, however, was to be expected, since frogs and other cold blooded animals are not poisoned by Cantharides.

Check for Experiment V.

1. A half grown *Rana pipiens* was fed about 0.5 gram *Mylabris* in water at 4 P.M. It received a similar dose at 9:30 A.M. the next day. In about two hours it was dead. It seemed impossible that the blister beetles had this effect because of the evidence to the contrary, in Vezien (1860), Meynier (1893) and others cited above.

2. A large adult *Rana pipiens* was fed 0.5 gram *Mylabris* in

water at 9:30 and 4:30. The next day the water in the jar in which it was kept was full of particles of the insects, showing that they passed through the alimentary canal rather rapidly, probably before any material was absorbed.

3. The same frog was then fed aqueous extract made by macerating 0.5 gram Mylabris in 4 cc. distilled water at 60° C. for 48 hours. The frog received 3 cc. of this solution every day for one week, and showed no ill effect at all, but was very active and finally did escape several weeks later.

4. Three Rana pipiens received 0.5 gram Mylabris in water. The next morning one had died, and was used in a subsequent experiment. The other two were fed the same amount again, and it passed out in four to six hours. This was repeated four times, making a total of six feedings for the two frogs; during the whole time they did not seem to be affected in the least. They were then killed and used in the same experiment as indicated above.

Experiment VI.

1. The frog from check for Experiment V, 1 and 2, was given to a rat, and for twenty-four hours it would not eat it. At the end of that time it was eaten, but, contrary to all expectation, the rat was not affected in the least.

2. The same rat was fed on two successive days the legs of the two frogs from Check for Experiment V, 3, which had each eaten 3 grams of cantharides. The rat suffered no ill effect then, or at any time following; it was not dissected, but was saved for future experiment.

Thinking that the rat in the two previous trials might possess a sort of individual immunity, the writer determined to try out the same experiment on different rats.

3. Two young white rats were selected, and each given one-half of a frog which had ingested six grams of cantharides in the preceding week. The alimentary canal of the frog was removed to obviate the possibility of there being any unchanged blister beetles present to affect the rats. As before, the rats ate the flesh, but showed no reaction.

Since the results in the preceding trials with indirect feeding of cantharides were all negative, it seemed useless to expect any effect from similar trials with Calendra; therefore these trials were dispensed with.

Experiment VII and Check.

1. Fifteen young chickens, approximately three weeks old, were put in a pen and divided into three lots of five each. Five were fed 0.5 gram Calendra, five an equal amount of Mylabris, and five reserved as controls. In all cases the insects were mixed with distilled water and fed with a large pipette. All the chicks were treated alike, and none showed any reaction that day or the following day. The second day after the feeding one chick of each lot was killed and dissected for comparison, but so far as could be observed, no pathological condition had resulted from the feeding.

2. Some of the flesh of each chicken was fed to three young rats, expecting, if the properties of cantharides as stated in Kobert and others were true, to cause the death of the rat which ate the Mylabris-fed chick. But, as in the frog-rat experiment, such was not the case: all three rats continued healthy and unaffected.

3. The four remaining Mylabris-fed chickens were each given one-half gram more of Mylabris, in an endeavor to see if there had not been sufficient given in the first trial to poison the flesh. One chicken was killed the next day and fed to the same rat as in Experiment VII, 2, but no result followed, although it was kept for a week before feeding it any other suspected material.

As before, since there was no result from this indirect feeding, it was not thought necessary to carry out similar experiments with Calendra.

Summary.

In summarizing the effects of the direct feeding of Calendra to mice, rats, frogs, chickens and rabbits, and the indirect feeding of frogs to rats and chickens to rats, there are three obvious conclusions which one might draw from the negative results: First, that C. granaria does not contain any poison; second, that their toxic element, if present, has no effect on the body through the digestive system because it is much less concentrated than in the true blister beetles; third, that the poison, if present, shows its activity in some other way than following ingestion, due to volatility or other cause. The evidence, however, would seem to incline one toward acceptance of the first view.

B. VESICATING EXPERIMENTS.

The preparation of all materials and solutions used for vesicating was governed by the methods indicated above in the section under "Methods of Extraction." Few attempts were made to extract crystals of cantharidin because of the lack of special training and equipment for such pharmaceutical work; but in all cases the preparations were made with reagents and processes suggested by the pharmacists previously cited.

Experiment I.

1. Some dried Calendra was rubbed on the skin of the inner arm, following which a slight redness developed, probably due to friction.

2. Check for Experiment I.

Some dried Mylabris was rubbed on the skin of the inner arm, but no burning or vesication resulted. This was to be expected, since the active element is contained in the body fluid of the insect, and is not capable of exercising its effect without a liquid medium.

Experiment II.

1. Five fresh Calendra were rubbed on the skin of the inner arm and allowed to dry there. No effect whatever was noticed.

2. Check for Experiment II.

One fresh blister beetle, Meloe angusticollis, was rubbed on the skin of the inner arm and after drying there, redness began to develop. After three hours the burning and redness seemed to intensify, until a small blister appeared.

Experiment III.

1. One-half gram of powdered weevils was macerated in 3 cc. distilled water for one week. The extract was filtered off at the end of that time, and was yellowish in color. A pledget of absorbent cotton was soaked in the cotton and bandaged on the wrist with

thin rubber, covered by gauze, to make an air-tight dressing. At the end of twelve hours the dressing was removed, and there was not the slightest effect on the skin.

2. Check for Experiment III.

One-half gram of Mylabris was macerated in 3 cc. distilled water for six days. The extract which was then filtered off was deep brown in color. It was bandaged on the wrist using cotton, rubber and gauze, and removed in two hours because of the severe pain the pressure of the bandage caused. Immediately the pressure was removed, a very large full blister formed, which contained approximately 2 cc. serous fluid.

Experiment IV.

1. One-half gram crushed Calendra was put in a test tube with 4 cc. distilled water and boiled down to 2 cc. Some of this yellowish brown fluid was put on cotton and bandaged on; after eight hours it was removed, and had not affected the skin in the least.

2. Check for Experiment IV.

One-half gram Mylabris was boiled in 4 cc. distilled water down to 2 cc. The brownish fluid was bandaged on as before, and in three hours the dressing was removed, and a large full blister formed.

Experiment V.

1. 300 Calendra macerated in chloroform for 36 hours, filtered and evaporated to a few drops. The fluid was soaked on lens paper and bandaged on. After eight hours the bandage was removed, but there was no effect on the skin, not even the

slightest suspicion of redness.

2. Check for Experiment V.

One-half gram Mylabris macerated in chloroform for 36 hours, filtered, soaked on cotton and applied on the inner side of the fore arm as before. In six hours a blister resulted similar to those in the other trials.

Experiment VI.

1. Ten fresh Calendra were ground to a coarse paste in pure glycerine (Stokes, 1914) andbandaged on the arm. After twenty-four hours of application there was no effect whatever.

2. Check for Experiment VI.

An equal amount of powdered Mylabris was mixed with pure glycerine, spread on lens paper and applied. After seven hours itching and burning began, and in two hours more it became so intense that the dressing was removed, and immediately a large painful blister was formed.

Experiment VII.

1. Some powdered Calendra was macerated in ether for twenty-five minutes, then the ether was distilled off (Carpenter, 1832) poured back on, redistilled, poured back and distilled for the third time. This time the ether was allowed to evaporate, and the retort was found to contain several cubic centimeters of yellowish, thick and not quite clear liquid. One-half of the liquid was soaked up on lens paper and applied to the arm for twelve hours, but had no effect whatever on the skin. The other half of the residue was allowed to evaporate, and very small crystals of a prismatic shape resulted, which were not comparable in form or

properties to crystallized cantharidin.

2. Check for Experiment VII.

The powdered Mylabris was macerated in ether for twenty-five minutes and distilled three times as in number 1. The inside of the retort was heavily coated with a greenish brown greasy substance, and a very small quantity of deep yellow fluid left. Both these substances, when applied to the skin, produced a blister at the end of three hours.

Experiment VIII.

1. Three grams of Calendra crushed and mixed with 2 cc. pure cedar oil, and placed in the oven at 55° C. for 96 hours. Filtered and applied, there was no effect on the skin after twenty-four hours.

2. Check for Experiment VIII.

Three grams of Mylabris mixed with 2 cc. cedar oil and kept at 55° C. for 128 hours. Then the liquid was filtered off, soaked on cotton and bandaged on. After two and one-half hours the pain necessitated removing the bandage, and in two hours more a large blister was fully formed. Due to the spreading of the oil the blister was the largest which had ever formed.

Experiment IX.

1. One-half gram Calendra macerated for one week in acetic ether, filtered and soaked on absorbent cotton. After eight hours of application there was no effect except a slight redness and peeling which was subsequently proved to be due to the acetic acid alone.

2. Check for Experiment IX.

One-half gram Mylabris macerated for one week in acetic ether, filtered and applied on absorbent cotton. In four hours a large blister had formed.

Experiment X.

1. One-half gram Calendra macerated in 3 cc. of 95 per cent alcohol. A yellow clear liquid resulted which produced no effect on the skin after twelve hours of application.

2. Check for Experiment X.

One-half gram Mylabris macerated in 3 cc. of 95 per cent alcohol for one week. This caused a blister at the end of five hours, with practically no pain.

Experiment XI.

1. Cantharidin "vinegar" (U. S. Dispensatory), one-half gram powdered Calendra added to 5 cc. 50 per cent glacial acetic acid, and heated in boiling water for one hour. The liquid, at the end of that time, was a deep greenish brown; cooled, filtered and added to 18 cc. slightly acetic alcohol. This liquid was painted on the arm, and immediately resulted in a bright pink area with slight burning while the alcohol was evaporating. The redness lasted fully an hour.

2. Since this was the first indication that Calendra had any irritating power whatever, it was determined to try a more concentrated extract to see if a blister might not be formed. 2 cc. of the liquid was evaporated down to a few drops and applied on cotton to the inner arm. Almost instantly a very severe burning was felt which became so intense that it was removed without being bandaged

on. The area covered by the soaked cotton showed a white wheal which gradually became inflamed, and surrounded by a wide inflamed band. Thinking to cause a blister if the cotton was left on longer, it was replaced, but the pain was so intense that it had to be removed after five minutes, and the whole surface of the inner arm became exquisitely painful, but no blister formed. After twenty-four hours a deep red sensitive spot remained, which peeled and acted in all respects like a rather severe acid burn.

3. The thought next presented itself that this reaction might have occurred simply from the acetic acid, which was present in rather concentrated form. A similar preparation was then made leaving out the insects, and painted on as before. Exactly the same results followed this application as with the _Calendra_-acetic solution. When a pledget of cotton soaked in the solution was applied for five to ten minutes the same pain resulted, and the same type of burn, proving that it was not the activity of the _Calendra_ which caused the burning.

4. Check for Experiment XI.

One-half gram _Mylabris_ was added to 5 cc. of 50 per cent glacial acetic acid, and heated in boiling water for one hour. The deep brown solution which resulted was applied on cotton without concentrating, to avoid if possible, any burning by the acetic acid. There was a slight burning at first, which gradually subsided, and in six hours a perfect blister had formed.

Experiment XII.

1. One-half gram of _Calendra_ was macerated in chloroform for 96 hours, filtered and distilled. The residue was evaporated down

to 1 cc. and mixed with 5 cc. CS_2. After evaporating, there were clear whitish crystals left, which blistered the skin after six hours of application.

Summary.

Summarizing the experiments on the vesicating effect of Calendra, the evidence that they do not possess such power is without any contradiction so far as my experiments have gone. Without exception the control preparation with Mylabris caused vesication, and since the materials used were the true solvents of cantharidin, it seems to follow that Calendra granaria possesses no cantharidin. If vesication ever resulted from this species, which is at least exceedingly doubtful, if not actually impossible, the causative agent was probably contained in the reagents used, and not in the insects themselves.

C. SUBCUTANEOUS INOCULATION.

The solution used in inoculating was prepared by mixing one gram of the dried Calendra with 8 cc. of distilled water, and boiling down to 4 cc. The resulting fluid was a muddy yellow; this solution was then diluted to 8 cc. with a salt solution double the strength of isotonic(or .0018 NaCl solution) making the final product isotonic (or .0009 NaCl solution). This was done so that there would be as little reaction as possible to the fluid itself, and any effect obtained could be attributed directly to the Calendra.

A control solution of the same concentration without the insects was made up at the same time. After the solutions were made they were sterilized in an autoclave, and injected with a sterile hypodermic syringe. The subjects used were rats and a mouse, and in all cases the inoculations were made subcutaneously in the lower left abdominal quadrant, where the skin had previously been exposed and cleansed with alcohol.

Experiment I.

1. At 2:40 P.M. an adult white mouse was inoculated with 1 cc. of the sterile solution of Calendra. It remained active the rest of the afternoon, and did not seem to be affected. During the two weeks following incoulation it was observed closely, but it continued normal.

2. Check for Experiment I. Inoculation with sterile isotonic solution likewise produced no results.

Experiment II.

1. Four young white rats were inoculated with 2 cc. each of the Calendra fluid prepared as above. They continued to be active and showed no ill effect. Observation extending over the following two weeks showed that they did not react unfavorably in any way.

Check for Experiment II.

1. Five rats were inoculated with the sterile salt solution, and observed carefully, but they showed no unfavorable reaction. One of each group was killed twenty-four hours later and dissected for comparison; but aside from the minute red point where the needle punctured the skin there was not the slightest evidence of the inoculation in either.

2. Four rats of the same age and condition were inoculated with 2 cc. each of a fluid prepared in exactly the same manner as above, save that *Mylabris* was used. The inoculation was begun at 2:30 P. M. and as soon as all four were incoulated they were returned to their cage. It was noted that these rats did not regain their normal activity as the rats inoculated with the other fluid did, and at the end of one hour they were all lying stretched out breathing rather convulsively. At 4:15 one rat was dead and a second one was lying on its side with strong muscular spasms of the hind legs. This twitching increased until the whole body was in constant convulsions, and death occurred at 4:30. The other two rats died in exactly the same manner at 4:40 and 4:50, approximately two hours after being injected.

They were all immediately autopsied, together with one of each of the *Calandra* and salt solution groups. The two last mentioned showed a slight redness at the site of the inoculation, with the surrounding tissues somewhat distended with fluid, but no inflammation; all the other organs seemed normal.

This picture showed a strong contrast to that of the rats inoculated with *Mylabris* solution. Here there was a great deal of inflammation both where the needle punctured the skin, and in the surrounding tissues. The most notable feature, however, was the way in which the entire area was congested with a clear yellowish jelly-like substance similar to coagulated blood serum, and adhering closely to the body wall. It might almost be described as a subcutaneous blister, covering a spot about three centimeters in diameter. The liver was also very much congested, and the lungs collapsed and bloodless. None of the other organs seemed very much affected; the poison probably acted too quickly to allow of penetration to all parts of the body, before the heart action was stopped.

These findings leave little doubt that Calendra contains no poison comparable in either amount or potency to that in Meloids.

VIII. *Leptinotarsa decemlineata*.

In view of the wide interest and discussion which the Colorado potato beetle (*Leptinotarsa decemlineata*) aroused in the period of 1850 to 1880, it will be well to keep in mind that this plant pest was only then migrating eastward from the Rocky Mountain region. Therefore it was a new and practically unknown enemy in the Central and Eastern United States, and naturally to be viewed with suspicion. Following is a quotation from an article by Dr. E. M. Hale, Professor of Medical Botany in the Hahnemann Medical School at Chicago (1869): "For what purpose this vile thing was created, except as one writer expresses it, that man might look upon it and the desolation it produces and still keep the Third Commandment unbroken, is yet to be discovered."

Following a morphological and biological discussion of the beetle, Dr. Hale says, "The bogus potato bug is not known to be poisonous, while a large amount of reliable testimony can be presented to prove that the Colorado potato bug is one of the most virulent poisons in the animal kingdom;" thus voicing what was probably the concensus of opinion regarding this insect at that time.

Walsh and Riley (1868) published a note by J. G. Irwin concerning poisoning by potato beetles, in which it was stated, "A friend of mine from Pierce County, Wis., tells me that the prairie chickens eat them readily, but that it sickens them, and some of them die in consequence; and people have quit hunting and eating the fowls. Domestic chickens seem to be affected in the same way, but turkeys will not touch them; though curious as it may seem, ducks and geese eat and thrive on them. A family within his knowledge, all ate of prairie chickens and were taken ill. His own son burned a lot of the bugs, and the fumes made him very sick. These things serve to confirm the poisonous nature of these bugs, and warn us to handle them carefully."

It may be well to note here that the above report as well as the following ones show an entire lack of accurate data. The cause and effect relationship, while it seems apparent enough, may be entirely false, and serve only to obscure the true state of affairs.

Dr. Hale (1869) divides poisoning by <u>Leptinotarsa</u> into three groups: (1) poisoning from inhaling fumes from insects placed in hot water; (2) by inhaling fumes from insects drying by dry heat; (3) from bruising them between the fingers or in the palms of the hands. No reports mention any such result as local vesication, but rather, results of a general systemic nature. There are ten more or less unauthenticated cases of poisoning included in his paper which all agree, however, in the symptoms presented by the patients. Briefly they are as follows: swelling of the whole body and especially the hands and face, blood-shot, protruding eyes, stupor, delirium, gastro-intestinal disturbances, vomiting, fever and often great prostration. Death occurred in four of these cases.

The above mentioned cases, however, were taken largely from newspaper items; thus it is obvious that their scientific value is slight, since the poisoning might have resulted from other and less apparent causes such as carbon monoxide poisoning from the hot coals on which the beetles were burned; but Dr. Hale has also included in his paper an experiment carried out by Dr. C. Ruden, of Joliet, Ill., which seems to be unassailable. In this experiment, the preparation used was "a saturated tincture" (which was not further defined); beginning June 1st the experimenter took 5 drops morning and evening. The 2nd of June the dose was increased to 10 drops, 15 drops the third, until on June 6th, he was taking 20 drops twice a day. During all this time the symptoms were very well marked, and were in general like those reported before; severe swelling of the entire body, face and eyes congested with blood, fever, gastro-intestinal pain, dysuria, intense weakness and stupor.

Dr. Hering, in Volume III Journal of Materia Medica, published a criticism of Hale's work in which he stated that "our doryphora is decidedly the most poisonous; nothing can be compared with it except the Argas of Miana in Persia, and the Coya in the valley Neyba in South America. It is altogether likely that more or less people will be poisoned by the Colorado beetle in the onward march to the Atlantic. I have witnessed several cases of the poisoning within the last three years, and can warn against handling the insect with over much freedom."

Dr. Hering suggests as an antidote the use of acetic, citric or other vegetable acid and stramonium, and summarizes his treatise by saying that "The Colorado Potato Beetle is one of the most violent poisons in nature. To handle, pick or kill the bugs mechanically, or by fire or boiling water has, in all cases, not only proved to be injurious, but in many cases it has caused long illnesses, sometimes terminating fatally. As these beetles are beautiful little things, and always appear the first year in small numbers they may attract attention, be caught and handled by children and others, and become the cause of diseases, even death, without the knowledge of parents or physicians."

Another antidote which Dr. Hering offers, which is not without its element of humor, is that "all who have to handle the beetle or come in contact with it accidentally, ought to rub their hands as soon as possible with earth, which is always at hand. They may afterward wash their hands with water, or not."

In looking through the reports of the State Entomologist of Missouri (C. V. Riley) from 1868 to 1876 there are many references to the poisonous qualities of these beetles, in which the trend of opinion runs from regarding them as violently poisonous to comparatively innocuous. In the first report, Riley says that no domestic fowl will touch a beetle as food, and that there are numerous cases on record where persons have been made violently ill by inhaling fumes from the scalded bodies, and have been confined to their beds many days in consequence.

In the third report published in 1870 Riley says that "chickens have learned to relish the eggs and have even acquired a taste for larvae" but there is no mention made of their poisonous qualities. The following year, we find that chickens have learned to eat even the adult, and will in fact, forsake all other food for potato beetles. The quail and the crow also eat the insect. Dr. Brown of the Indiana Horticultural Society even goes so far as to advise turning the chickens loose in an infested field, and raising the young there. It seems fairly obvious then, that the beetles do not poison the chickens, and that the earlier reports were exaggerated, if not entirely unfounded.

In the 7th annual report, 1875, the controversy is again revived by Prof. T. J. Burrill of the Illinois Industrial University, when he makes a flat statement to the effect that the insect is not poisonous; his support for the statement being that he had rubbed juice from the mashed insects into a flesh cut, and had accidentally squirted some into his eye, and had not had any injurious effects from either. Immediately a "certain sarcastic Chicago professor" (possibly Dr. Hale) replied that he could "fix up a decoction from the dead beetles that would cause a vacancy in the chair of Horticulture in the Illinois Industrial University if Prof. Burrill inhaled it."

Riley then goes on to say that three cases of poisoning had been reported to him by people of undoubted veracity and good judgment, which he explains by saying that though many people are immune from the poison, there is no doubt that, with blood in "certain bad conditions" poisoning may result. The poison seems to be of a very volatile nature, and all serious cases of illness have resulted from burning or scalding large quantities at a time, with production of swelling, pain and nausea in the victim.

In the 8th Annual Report there is included a very interesting series of experiments carried out by Grote and Kayser, 1876, which were read before the American Association for the Advancement of Science. Following are some extracts: "A quantity of bugs were collected from unsprayed fields and submitted to distillation with salt water at an increased temperature. About 4 oz. of liquid were obtained from 1 quart of the beetles; it was perfectly clear; emitted a highly offensive smell, and had an alkaline reaction.

"An equal quantity of bugs were used to prepare a tincture made by condensing pure absolute alcohol on the live insects, and allowing it to remain 24 hours. The liquid was then evaporated at a gentle heat, leaving a tincture of acid reaction, brown in color, and not unpleasant in odor. These two liquids were then introduced in 0.5 cc. doses into the stomachs of two frogs, but neither had the slightest effect. Next, two other frogs were submitted to a hypodermic injection of the solutions into the hind legs. The injection of the distilled liquid produced no result, while the alcoholic injection caused paralysis of the leg and subsequent death in about thirty minutes." The check experiment with plain salt solution showed no result. However, the decision was reached that the amount of animal acid was so small that the bugs are not poisonous.

The experimenters conclude that the reported cases of poisoning result from the arsenic used in destroying the insects, or from carbonous oxide (CO) produced by incomplete combustion when large amounts of beetles are introduced into a fire, but admit that the results reached by experiment are not convincing and need further substantiation.

Riley goes on to criticize the experiments and brings out some points which are well merited: namely, that the reagents used to extract the poison may not be the proper ones, and may only serve to coagulate it instead of extract it, and second, that the effect of the poison may differ vastly on cold and warm blooded animals.

In this unsatisfactory state, then, the question is left, with insufficient experimental data to either prove or disprove the accusations. In 1906 Kobert, evidently considering the amount of evidence sufficient, includes Leptinotarsa decemlineata in his list of poisonous Chrysomelidae.

In the case of this beetle the experimental work done by the writer may be divided into four parts: the feeding experiments, using the same kind of animals as before; vesicating experiments, performed always with the writer as subject; inhalation experiments on rats; and finally, the subcutaneous inoculation experiments on rats and chickens. In every case a control was used, as indicated.

A. FEEDING EXPERIMENTS, LEPTINOTARSA.

The preparation of material in all cases was as nearly parallel with the preparation of Calendra granaria as could be made, considering the difference in size, structure and habits of the two insects. The potato beetles were always collected from an unsprayed field, dried by using the dry heat of the oven or a large drying chamber, and ground to a powder. This powder was then fed to the animals dry, mixed with distilled water to a coarse paste, or boiled with distilled water to make a concentrated extract.

Experiment I.

1. Three young rats were fed 1 gram each of the powdered insect mixed with water to facilitate feeding. They showed no ill effects whatever during close observation for one week following the feeding.

2. The same three rats were fed 2 cc. of an extract made by

boiling 1 gram Leptinotarsa and 15 cc. distilled water down to 6 cc. They showed not the slightest effect.

3. Two other rats were each fed 2 grams of the insects after 24 to 48 hours of starvation. They were not affected unfavorably.

Check for Experiment I.

1. One rat from Experiment I, number 3, was dissected 18 hours after feeding, at the same time as a normal control rat. Comparison showed no gross differences between the two individuals.

Experiment II.

1. Five adult Rana pipiens were each fed 1 gram of powdered Leptinotarsa in a little water. As in the Calendra feeding, the insects passed through the alimentary tracts in four to six hours, so the feeding was repeated every day for three days, making a total intake of 4 grams apiece. The frogs were not affected, as far as could be observed during the two following weeks.

Experiment III.

1. Two frogs from the foregoing Experiment were fed to two rats from Experiment I, 3, after first removing the alimentary tracts of the frogs. The rats ate the frogs after 24 hours of starvation, and were not affected in any way.

Experiment IV.

1. Six three-week-old chicks were each fed 1 gram of Leptinotarsa in just enough water to make a paste. They were observed carefully but seemed to suffer no ill effects.

2. Six chickens of the same brood were also kept in this run and treated exactly the same, except that they were not given

the insects. Four days after the feeding, one control chick died. The weather turned cold and damp at this time and resulted in the chickens, both experimental and control, dying off rapidly, since they were not provided with a brooder to keep them warm. At the end of nine days after the feeding, four of the six Leptinotarsa chicks and five of the controls had died. This death rate, however, does not seem to indicate that the chickens fed on beetles were poisoned, since those unfed died also. But since the results were not conclusive, the experiment was repeated.

3. Five three-week-old chicks were each given 1 gram of the powdered beetles on three successive days. Although observed carefully for two weeks, no symptoms could be detected, and none died.

4. The five control chickens in this repetition also remained healthy, and none died during two weeks, so the experiment was terminated.

Experiment V.

1. The chickens from Experiment IV, 4, were each fed 0.5 gram of powder made from dried larvae of the potato beetles. Since they showed no effect the trial was repeated twice, making a total intake of 1.5 grams apiece, from which the chickens did not apparently suffer at all.

B. VESICATION EXPERIMENTS.

In this series of experiments the preparations were in all cases similar to the preparations of Calendra granaria. The only differences which occurred were that fewer individuals were used because of their large size; the elytra of these beetles were removed to aid in more rapid drying out and the preparing of a finer powder than would otherwise be possible. Two solutions were also made from dried larvae. The beetles were always collected in unsprayed fields, to eliminate the possibility of getting a result from some other substance than those normally present in the beetle.

Experiment I.

1. Dried and fresh adult beetles were rubbed on the skin of the inner arm with no appreciable effect.

2. Result of check experiment as in <u>Calendra</u>, Experiment I,2.

Experiment II.

1. One-half gram dried beetles was added to 6 cc. distilled water and boiled down to 3 cc. Bandaged on with gauze and rubber and allowed to remain for eight hours. No effect.

2. Check for Experiment II as in <u>Calendra</u>, Experiment IV,2.

Experiment III.

1. A paste made of dried beetles in distilled water was spread on lens paper and bandaged on. Removed after nine hours with no result.

2. Check as in <u>Calendra</u>, Experiment III, 2.

Experiment IV.

1. One adult beetle thoroughly mashed in pure glycerine spread on lens paper, is applied. After twenty-four hours it was removed and showed not the least effect.

2. Check as in <u>Calendra</u>, Experiment VI, 2.

Experiment V.

1. One-half gram of powdered beetles macerated in chloroform for 96 hours. Filtered, soaked on cotton and applied for

twelve hours. Slight brownish discoloration of the skin, but no burning or other reaction.

2. Check as in Calendra, Experiment V, 2.

Experiment VI.

1. One gram of powdered Leptinotarsa was macerated for thirty minutes in ether, then distilled. The distillate was condensed and poured back over the insects twice, and the third time allowed to escape. There was a very small amount of oily yellowish brown fluid left in the retort, which was soaked on lens paper and bandaged on. After twenty-four hours there was not the slightest effect on the skin.

2. Check as in Calendra, Experiment VII, 2.

Experiment VII.

1. One gram Leptinotarsa was macerated for one week in acetic ether. The fluid which resulted was yellowish brown, and after twelve hours of application caused no reaction on the skin save a slight brownish discoloration.

2. Check as in Calendra, Experiment IX, 2.

Experiment VIII.

1. One gram Leptinotarsa was macerated in 95 per cent alcohol for one week. A clear yellow fluid resulted which did not affect the skin after twenty-four hours of application.

2. Check as in Calendra, Experiment X, 2.

Experiment IX.

1. One gram Leptinotarsa was macerated in cedar oil for one week. Application on the skin of the inner arm was without result.

2. Check as in _Calendra_, Experiment VIII, 2.

Experiment X.

1. One gram _Leptinotarsa_ was macerated in 95 per cent alcohol for one week, filtered and thoroughly mixed with CS_2. After allowing to evaporate there was a yellowish oily scum left. This was applied to the wrist on lens paper but had no effect after ten hours.

2. Check as in _Calendra_, Experiment XII, 2.

Experiment XI.

1. One gram powdered larvae macerated in chloroform for six days. The liquid was clear yellow and similar in odor to the chloroform solution of the adults. It had no effect on the skin.

Experiment XII.

1. One gram powdered larvae macerated in 95 per cent alcohol for one week. This fluid was also inert when applied on the arm.

The results of the previous experiments are so uniformly negative, compared to the uniformly positive results of the control experiments with _Mylabris_, that there can be little doubt that _Leptinotarsa decemlineata_ contains no cantharidin, or it would have been effective in the various solutions prepared with its natural solvents.

C. SUBCUTANEOUS INOCULATION EXPERIMENTS.

The solution used in these experiments was prepared in exactly the same manner as the fluid in Section C of the _Calendra_ experiments, namely 1 gram of insects to 8 cc. of distilled water, boiled down to 4 cc. and diluted with .0018 NaCl solution, making 8 cc. of isotonic solution. This was autoclaved and injected as previously outlined.

Experiment I.

1. Five adult white rats were tied in position and the hair shaved from a space about the size of a dime. After sponging off the skin with 95 per cent alcohol, 2 cc. of the sterile fluid was injected with a sterile syringe. In every case the fluid formed a large bulge under the cutaneous layer, andwas gradually absorbed in the course of one hour. Although the rats were closely observed for the next ten days, no unfavorable reaction could be detected.

2. Check with sterile salt solution. Twenty-four hours after inoculation one rat of the previous group was killed and dissected in comparison with one of the five which had been inoculated with pure sterile isotonic salt solution. Both showed identical conditions in the area surrounding the puncture, and in the adjacent tissues and organs, as indicated in Calendra, C, Experiment I.

3. Check with inoculation of Mylabris solution in rat as indicated in Calendra, C, Experiment I.

Experiment II.

1. Four young chickens were tied in position and a patch of skin sponged off with 95 per cent alcohol. Then they were each injected with 2 cc. of solution prepared as in Experiment I. The fluid was absorbed in less than one hour and seemed to have no effect on the chickens.

2. Check with sterile salt solution. One of the chickens was killed and dissected in comparison with one of the four chickens inoculated at the same time (twenty-four hours previously) with pure sterile isotonic salt solution. Both showed the same condition in the area surrounding the puncture and the adjacent organs, as indicated in Calendra, C, Experiment I.

3. Check with inoculation of Mylabris solution as in Calendra, C, Experiment I.

The experimental inoculation with Leptinotarsa decemlineata shows beyond question that the solutions employed contain no toxic element similar to that found in the Mylabris solution. That these results are not exactly in agreement with the inoculation experiments of Grote and Kayser (1876) cited above, may be explained possibly by the fact that those workers obtained positive results only with the alcoholic tincture; which indicates that their effect was due rather to the alcohol than to the insect derivatives contained in it.

Experiment III.

1. In the inoculation experiments of Grote and Kayser, it seems fairly evident that there was some poisonous substance injected, or the frogs would not have died. Yet death occurred only following the injection of alcoholic solution of Leptinotarsa, and not from the aqueous solution. Therefore it seems reasonable that it was the alcohol which caused the death, and not any property of the insects. Accordingly, six adult frogs were selected and two injected in the hind leg with 2 cc. of 95 per cent alcohol, two with 2 cc. of Leptinotarsa in 95 per cent alcohol, and two with

2 cc. of an aqueous solution of <u>Leptinotarsa</u>. Immediately the four frogs which had ~~not~~ received the alcohol became paralyzed and inert, and a few hours later were very much puffed up. When they were prodded there was little response, and if they were turned on their backs it took several hours before they could regain their normal position. Two of the four frogs died within twelve hours, and the other two retained a little life for two days following, and then died. During this time the frogs which had received the aqueous solution of the beetles were lively and normal.

One of each group was dissected for purposes of comparison, but little could be distinguished except that the frogs which had received the alcohol had the outer layer of skin very much distended, and showed some inflammation of the surrounding tissues.

This experiment proves fairly conclusively that the death of the frog is caused by the alcohol and not by the insects.

2. The two frogs which had been inoculated with aqueous solution of <u>Leptinotarsa</u> both remained normal and unaffected.

D. <u>INHALATION</u> <u>EXPERIMENT</u>.

Riley, Hale and others have mentioned the fact that one method by which individuals were poisoned was inhalation of steam from beetles thrown into boiling water, or fumes from insects under dry heat. Therefore the writer determined to try several experiments to get a similar result, if possible.

Experiment I.

1. Approximately 1 quart of beetles were spread out on flat trays to dry in a hot box 4x4x4½ feet, with a temperature of 60° C.

The box was tightly closed for several hours, and became filled with acrid, unpleasant fumes. The box was then opened and the fumes breathed by the writer for twenty minutes. The experiment was repeated on the following day. While the odor was intensely unpleasant, there were absolutely none of the symptoms developed which were reported in the above mentioned papers.

Experiment II.

1. Ten grams of fresh Leptinotarsa were mashed and put in a retort with distilled water and kept at a slow boil. The fumes given off were similar to those in Experiment I, and were inhaled for two periods of thirty minutes each, with no effect beside an unpleasant taste which remained in the mouth for several hours.

Experiment III.

1. A young rat was put in a small wire container closely covered with a damp towel, and the steam from the retort directed into the cage through a small opening. At no time did the temperature in the cage rise above 35° C. After three-quarters of an hour the rat showed no effect. The trial was repeated on the three following days, and the rat continued unaffected.

2. Check for Experiment III.

A young rat was put in the same cage and pure steam directed into it for the same length of time. Here also, there was no reaction.

IX. Miscellaneous Experiments.

Experiment I.

1. The statement has occurred in several different papers that the fluid from a blister raised by application of blister beetles is poisonous. It has been alleged that this fluid contains enough cantharidin to vesicate, or if taken internally, will act like any other preparation of cantharidin. Accordingly some fluid from a blister was soaked on absorbent cotton and bandaged on the wrist with rubber and gauze. After twelve hours there was no effect on the skin.

2. A very large blister was raised with a chloroform solution of Mylabris, and the fluid drained into a sterile vessel. It was then drawn up into a sterile hypodermic needle. Two young rats were prepared as in the other inoculation experiments, and each injected with 2 cc. of the serum. In the course of one hour the fluid was absorbed, and the rats showed no reaction.

Thus it is concluded that the serous fluid does not contain any cantharidin.

Experiment II.

1. Several entomologists of this University have reported that while they were doing night collecting around electric lights, there was a small black beetle which caused intense smarting and burning when it flew against the face and in the eyes, the eyes becoming severely bloodshot. Several specimens were brought in to be tried out. They were small Chrysomelids of the genus Haltica, which commonly hover in clouds around lights. These were dried, macerated in chloroform, and the fluid applied to the wrist,

without obtaining any effect.

2. Some of the juices of the fresh beetle were rubbed into the eye without giving any such reaction as mentioned above. It was therefore concluded that these were not the irritating insects.

Experiment III.

In Kobert (1906) a species of Coccinelid belonging to the genus Adalia was listed as poisonous. The common Adalia bipunctata which occurs abundantly in this region was collected, dried and prepared in chloroform. It had no irritating action on the skin.

Experiment IV.

After Fabre, in his volume on "The Life of the Caterpillar" cited above, it was determined to try out the fluids ejected from the body of different insects at the end of the pupal period, to see if ulceration and sloughing of the skin resulted from all species as he claimed.

1. Accordingly, six pupae of the variegated cutworm, Peridroma margaritosa, were put on clean filter paper under glass just as they were ready to emerge. The yellowish fluid ejected after their emergence was deposited on the filter paper and allowed to dry. It was then soaked off in ether and the ether solution applied on lens paper as in Fabre. There was no irritation or other reaction after twelve hours of application.

2. Ten pupae of Tenebrio molitor were placed on filter paper and the fluids ejected were soaked into the paper. The paper was placed in ether as before and then the solution bandaged on. There was no effect on the skin.

Whether one is to judge by these results that only the species Fabre used have this effect, or that his results were obtained through some peculiar sensitivity in himself, must remain a question until time can be given to carry out his experiments more minutely.

Experiment V.

The Meloidae in this immediate vicinity do not seem to be very numerous, but the four species that were collected during the past summer were tested out. They were Epicauta pennsylvanica, Epicauta vittata, Meloe angusticollis and Macrobasis unicolor. The whole insect was mashed in a little chloroform, macerated for twenty-four to ninety-six hours, and the solution applied on cotton. A perfect blister was obtained in each case, the time length varying from three to nine hours.

Experiment VI.

Lamson (1914) has reported that the rose chafer, Macrodactylus subspinosa is toxic to young chickens, and that their death is not caused as has heretofore been believed, by a cropbound condition. He proved this by feeding young chicks an aqueous extract which resulted in their death with the same symptoms and in the same time as in the ingestion of the whole insect. By way of checking up Lamson's results and also adding to them if possible, several series of experiments were attempted. A number of solutions similar to those of Mylabris, Calendra and Leptinotarsa were made up and applied to the skin, but none of them caused vesication or any other reaction. Next an isotonic solution was made, sterilized, and inoculated into four rats and four chickens, expecting that the results would correspond to those of Lamson, who caused the death of both chickens and rabbits by intravenous injections. But these subjects were not affected in the least, nor did any deaths result from heavy feeding of the chafers to chicks and rats. Therefore, one would be forced to conclude either that Lamson's results were obtained through some unusual combination of circumstances, or that the specimens used by the writer were different in some way from Lamson's. Since Macrodactylus subspinosa is not found here, the specimens were sent from Pennsylvania, and were not used for some months after their death. If the poison is volatile this would explain the difference in results, since Lamson presumably used the fresh insects. Further experiment was postponed by the writer until such a time as fresh insects could be obtained.

X. Bibliography.

ARNAUD and BRONGNIART, 1888. Sur une Cigale vèsicante de la Chine et du Tonkin. Comptes rendus de l'Academie des Sciences, cvi, p. 607.

BASSI, R., 1893. Eczema vesicoloso labiale e nasale del cavallo causato in maniera indiretta par Blaps nero. Il Moderno Zooaitro, iv, p. 63.

BEAUREGARD, H., 1890. Les Insects Vésicants. Paris, Ancienne Librairie Germer Baillière et Cie. Felix Alcan, Ed. 465 p.

____________ 1885. Seat of the Active Principle in Vesicating Insects. Pharm. Journ., xv, p. 873.

____________ 1887. Réchérches sur les Insects vésicants. Jour. de l'Anat. Phys., xxiii, p. 124-163.

BEGUIN, M., 1874. Histoire des Insects qui peuv. être employeés comme vésicants. Paris, Soissons, Berthaut & Couturier. 116 p. 4°.

BERTHOUD, P., 1859. Étude sur la cantharidine offic. Thèse de l'ècole de Pharmacie, Paris.

BLANCHARD, R., 1890. Traité de Zool. Medicale, vol. ii, p. 543-573. Paris, Librairie Jermer Baillière et Fils.

BOND, R., 1880. Potato Bug Poisoning. Med. Bull. of Phila. ii, p.14.

BRETTONEAU, 1828. Journal de Pharmacie, xiv.

BROWN, 1871. Trans. Indiana Hort. Soc., p. 81.

CARPENTER, G. W., 1831. Notice of the Vesicating Principle of Cantharides. Am. Jour. Sci. and Arts, xxi, p. 69-70.

CHALMERS, A. J. and KING, H. H., 1917. Blister Beetles as a Public Nuisance. New Orl. Med. and Sur. Jour., lxx, p. 445-455, 1 pl.

CORNIL, V., 1880. Recherches histologiques sur l'action toxique de la cantharidine et de la poudre de Cantharide. Jour. de l'Anatomie, xvi, p. 566.

CORNIL et TOUPET, 1887. Sur la karyokinèse des cellules epithél-iales et de l'endothélium vasculaire observée dans la rein à la suite de l'empoissonnement par la cantharidine. Arch. de Physiol. xix, p. 71.

COURBON, A., 1855. Observations sur les Coléopteres vesicants des environs de Montevideo. Comptes rendus de l'Acad. des Sciences, xli, p. 1003-1006.

CUENOT, L. 1890. Les insects vesicants, et le role biologique de la cantharidine. Bull. Soc. Ent. France, xv, p.126-128.

DA SILVA, M. P. 1912. Le Paederus columbinus est Vesicant. Arch. de Parasitologie, xv, p. 431, 1 pl.

DRAGENDORFF, 1867. Pharm. Zeitschr. f. Russl., Bd. 3, p. 165, and Bd. 6, p. 1, 143.

EYSELL, A. 1913. Coleoptera, in Mense's Tropenkrankheiten. 2nd Ed. Leipzig. p. 247-248.

FABRE, J. H. 1916. The Life of the Caterpillar, transl. by A. T. de Mattos. Dodd, Mead & Co., N. Y. p.128-186.

FAHNESTOCK, L. 1879. Valuation of Blistering Beetles. Amer. Jour. Pharm.,li, p.296.

FARINES, 1826. Jour. de Pharm. xii.

FERRER, L. 1859. Essai sur les Insects vesicants. These de l'Ecole de Pharmacie. Paris. p.1-60.

FUMOUZE, A. 1867. De la Cantharide officinale. These de l'Ecole de Pharmacie. Paris.

__________ 1869. Bull. Soc. Ent. France. p.30.

__________ 1888. Sur l'Huechys sanguinea (Cicada sanguineolenta d'Olivier). Comptes rendus de l'Acad. des Sciences. cvi, p.759-762.

GALIPPE, 1876. Etude toxicologique sur l'empoissonment par la cantharidine et par les preparations cantharidiennes. Paris.

GALLAGHER, B. A. 1920. Poisonous Qualities of the Rose Chafer. Jour. Am. Vet. Med. Assoc. Wash. D.C., lvii, N.S. x, No. 6, Sept., 1920. p.692-695.

GOOSSENS, T. 1881. Des chenilles urticantes. Ann. Soc. Ent. de France. 1881, p. 231-237.

__________ 1886. Des chenilles vesicantes. Ann. Soc. Ent. de France, vi, p.461-464.

GREENISH and WILSON. 1898. The Pharmacy of Cantharides. Pharm. Jour. 1898, 6-7, p.255-259, and p.261-262.

GROTE, A. R., and KAYSER, A. 1876. Are Potato Bugs Poisonous? Amer. Assoc. Adv. Sci. 1876. Included in Report of State Entomologist of Missouri, No. 8. (C. V. Riley).

HALE, E. M. 1869. The Colorado Potato Beetle. Transactions Homeop. Med. Soc. State of N. Y. viii, p.142-169. 3 pl.

HENRY and BONASTRE. 1827. Recherches analytiques sur les charancons du ble. Journal de Pharmacie, xiii, p. 539.

HENTZ, N. M. 1821. A Notice Concerning the Spider, whose Webb is used in Medicine. Jour. Acad. Nat. Sci. Phila. ii, p.53-55.

HERING and MARTIN. Colorado Potato Beetle. Jour. of Materia Medica, iii, No. 1.

HERRICK, G. W. 1914. Insects Injurious to the Household. New York, MacMillan & Co. p.258.

IRWIN, J. G. 1869. Poisoning by the Potato Beetle. Amer. Entomologist, i, p.118.

KOBERT, R. 1906. Lehrbuch der Intoxikationen. 2 Volumes. Stuttgart, Verlag von Ferdinand Enke. Vol.ii,p.435-444.

LAMSON, G. H. 1911. Rose Chafers Poisonous to Young Chickens. Connecticut Farmer, July 1, 1911, No. 10.

__________ 1913. Rose Chafers are Poisonous to Chickens. Connecticut Farmer, July 12, 1913, No. 7.

__________ 1914. Poisonous Effects of Rose Chafers on Chickens. Jour. Ec. Ent. viii, p.547-548.

__________ 1916. Poisonous Effects of the Rose Chafer on Chickens. Science, n.s., vol.43, p.138.

LAVINI and SOBRERO, 1832. Jour. de Pharmacie, viii, p.467.

LECLERE, 1835. These de l'Ecole de Medecin. Paris.

_____ 1828. Ann. Sc. Nat. xiii.

LEIDY, J. 1869. Seat of the Vesicating Principle of L. vittata. Am. Jour. Med. Sci., vols.39-40, p.60-62.

LISSONDE, L. 1869. De la Cantharide. These de l'Ecole de Pharmacie. Paris.

MEYNIER, J. 1893. Empoissonment par le chair de grenouilles infectees par des Insects du genre Mylabris. Arch. de med. mil. xxii, p.53-56.

MILLS, W. 1835. Observations upon the Corn Weevil. Trans. London Ent. Soc. i,p.242.

MITOUART et BONASTRE. 1827. Sur Calendra granaria, son analyse. Jour. de Pharmacie, xiii, p.508.

MOUFET, T. 1658. The Theatre of Insects or Lesser Living Creatures, in Topsel's History of Beasts. London, 1658. p.660-665.

NEUTWICH, 1870. Inefficacy of Young Cantharides. Pharmaceutical Journal (3) 1,p.158.

PERIER, L. 1880. Note histologique sur la Cantharide. Bull. de travaux de la Soc. de Pharm. de Bordeaux, mai-juin, 1880.

PORCHER, F. P. 1863. Resources of the Southern Fields and Forests. Pub. and prep. by order of Surgeon General. Richmond, Va. Walker, Evans and Cogswell, Charleston. p.397-400.

RAILLET, A. 1895. Insectes Coleopteres. Traites de Zool. Med. et Agricole. 2nd Edition. Asselin et Houzeau, Paris. p.886-901.

RILEY, C. V. 1868. Note on Leptinotarsa decemlineata. First Annual Report, State Entomologist of Missouri. p.115-116.

__________ 1870. Remarks on Chickens feeding on L. decemlineata. Third Annual Report, State Entomologist of Missouri, p.100.

__________ 1871. Chickens feeding on L. decemlineata. Fourth Annual Report, State Entomologist of Missouri, p. 16.

__________ 1873. Notes on Leptinotarsa decemlineata. Fifth Annual Report, State Entomologist of Missouri, p.125-126, 131, 134.

__________ 1875. Is it Poisonous? (L. decemlineata). Seventh Annual Report, State Entomologist of Missouri, p.6-9.

__________ 1876. Notes on L. decemlineata. Eighth Annual Report, State Entomologist of Missouri, p.10-13.

__________ 1878. On larval characteristics and habits of blistering beetles belonging to genera Macrobasis and Epicauta, with remarks on other species of the family Meloidae. Trans. St. Louis Acad. Sci. iii, p.544-562.

ROBIQUET, M. 1811. Experiments sur la Cantharides. Ann. de Chimie, lxxvi, p.302.

RODHAIN and HOUSSIAU. 1915. Dermatite vesiculeuse saisonniere produite par un Coleoptere. Bull. de la Soc. de Path. Exotique, viii, p.587-591.

ROSS, P. H. 1916. Dermatitis due to the Secretion of a Beetle in British East Africa. Jour. Trop. Med. and Hyg. xix, p.202.

SAUNDERS, W. and REED, E. B. 1871. Report on Colorado Potato Beetle. Can. Ent. iii, p.41-51. 9 ill.

SAUNDERS, W. 1876. Notes on Cantharides. Can. Ent. viii, p.221-229

TISSERAUT, H. 1886. Accidents provoquees chez la cheval par Blaps mortisaga. Jour. de Med. Vet. et de Zootechnie, 1886, p.25.

UNITED STATES DISPENSATORY, 20th Edition. Remington and Wood.

VEZIEN, M. 1860. Cystite cantharidienne causee par l'ingestion de grenouilles. Recueil des mem. de med. mil. iv, p.457.

WALSH, B. D. and RILEY, C. V. 1862. Grain weevils. Jour. Ill. State Agr. Soc., Jan. 1862.

__________ 1862. Grain Weevils. Trans. Ill. State Agr. Soc. v, p.484.

__________ 1869. Poisonous Flour. Amer. Entomologist, i, p.179.

__________ 1870. Anis seed and Grain Weevils. Amer. Entomologist, i, p.41-45.

__________ 1871. Poisonous Qualities of the Colorado Potato Beetle. Amer. Entomologist, ll, p.85-86.

__________ 1875. Is the Colorado Potato Beetle Poisonous? N. Y. Weekly Tribune, Feb. 17, 1875.

WARD, A. R. and GALLAGHER, B. A. 1920. Poisonous Qualities of Macrodactylus subspinosa. Diseases of Domesticated Chickens, 1920.

WARNER, W. R. 1856. On the Medical Value of C. vittata and M. cichorii as compared with C. vesicatoria. Amer. Jour. Pharm. xxviii, p.195.

WHITE, W. H. 1909. Properties and Preparations of Cantharidin. White's Materia Medica and Therapeutics. Wilcox, 5th Edition. p.522,665,697.

WITTHAUS, M. L. 1911. Toxicology, N. Y.