the frog was performed by an intravenous technique. Various types of control experiments are described.

2. Aged tinctures were always found to be less potent by assay in both species; however, the greater reduction in potency was usually observed in the frog.

3. Deterioration of tinctures could not be correlated with increased "p_H;" on the contrary the tinctures most stable by cat-assay were shown to yield the highest glass-electrode potentials.

4. A sample of digitoxin in absolute alcohol lost about half of its toxicity toward both the frog and the cat after a few months. There was no further deterioration during the period of observation (59 weeks).

5. Frogs were found to be least susceptible to ouabain in the late summer (August).

6. The results of assays in 601 cats indicated that the distribution of susceptibility is probably normal.

We wish to thank Mr. F. A. Upsher Smith whose generous support made this investigation possible.

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AMINO-ALCOHOLS. IX. BIOLOGIC ASSAY OF PROPADRIN AND EPHEDRINE.*[1]

BY THOMAS S. GITHENS, M.D.

The physiologic assay of phenylpropanolamine (propadrin) and phenylpropanolmethylamine (ephedrine) is a problem which still awaits completely satisfactory solution. Although the qualitative action of ephedrine has been elucidated fairly thoroughly, and it is known to act on most, if not all, of the structures innervated by the autonomic system, none of these actions lend themselves to accurate quantitative analysis. Its actions on each of these structures differ so widely, quantitatively at least, from one animal to another and all are so markedly influenced by slight experimental differences which are not readily analyzed, that it is not possible to obtain quantitative determinations by comparing the action on one animal directly with that on another.

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* Presented under title "Bioassay of Propadrin Solutions," by T. S. Githens, James C. Munch and W. H. Hartung, before Scientific Section, A. PH. A., Toronto meeting, 1932.

[1] From The Mulford Biological Laboratories, Sharp and Dohme, Glenolden, Pa.

The effects which would seem to be most readily measured are those on the pupil, on isolated organs, such as the uterus and intestines, the dilation of the bronchi, which can be shown on the lung *in situ*, or isolated, and the pressor action, which is due to stimulation of the heart, associated with vaso-constriction. (Chen and Schmidt, *J. Am. Med. Assoc.*, 87 (1926), 836.) Studies by many authors of the effects on isolated organs and bronchi, have not shown such regularity as would recommend them for quantitative assays.

Swanson (*J. Pharmacol. & Exper. Therap.*, 38 (1930), 327) reports his findings on bronchodilatation from perfusion of the isolated lungs of 10 rabbits, 12 cats and 15 dogs. He used Sollmann and Van Oettingen's method, counting the bubbles per minute escaping in a Mariotte bottle. He does not report his results in detail, but gives a table showing the average increase of flow from 24 mg. added to the perfusion fluid, as 5 drops per minute. His published tracings show increases of flow varying from 3 to 9 drops per minute from a dose of 20 mg. This 300 per cent variation, which was even greater in our own work, illustrates the difficulty of obtaining quantitative results by this method.

Most biologically assayed drugs acting on the autonomic system, such as epinephrine and pituitary extract, are assayed by comparing the results obtained on a particular animal, or piece of tissue, with the effect produced on the same test object by a standard solution of the material being tested. In the case of ephedrine, however, this method is rendered illusory by the fact that its effect is reduced or altered by previous administration, so that whichever sample is first employed will appear to be the stronger. This reduced effect on repeated administration (tachyphylaxis) is true not only of its pressor action, on which it has been most carefully studied, but also of its effects on isolated tissues.

If a dog or cat be given by vein a dose of ephedrine which will cause a marked rise of pressure (*e. g.*, 0.5 to 2 mg. per kilo), and the same dose be repeated, even at intervals of several hours, the second dose will cause less rise than the first, the third still less, and eventually a fall may result. This lessened pressor effect probably depends mainly on the cardiac action of the drug, and is believed by Müggе (*Arch. exptl. Path. Pharmakol.*, 165 (1932), 230) to result from the fact that the flow through the coronary vessels is increased by the first injection and lessened by later doses. There is, however, no fixed relation between the rise caused by the first and by subsequent doses. This fact makes it impossible to "standardize" the test animal by giving a definite dose of a standard preparation and to base the assay on the relation between the rise caused by this and the rise following administration of a solution of unknown strength. If the doses are much smaller than that which gives a maximum response, this tachyphylaxis tends to become less with repetition of the dose, and eventually subsequent doses may give substantially identical effects, although the response is never as regular as it is with repeated injections of the same dose of epinephrine. Thus, Tainter (*J. Pharmacol. & Exper. Therap.*, 36 (1929), 569) injecting several cats and dogs with repeated doses of 1 to $1^1/_2$ mg. per kilo, at intervals of an hour, found the following average rises of pressure expressed in percentage of the original blood pressure; 1st injection, 30 per cent; 2nd injection, 26 per cent; 3rd injection, 22 per cent; 4th injection, 19 per cent; 5th injection, 19 per cent.

Pittenger (JOUR. A. PH. A., 17 (1928), 634) suggested that the mydriatic

action might be utilized in quantitative analysis, but apparently no work along this line has been published. In a quantitative study of the effect of 2 drops of 10 per cent ephedrine in a series of normal caucasians, Chen (*J. Pharmacol. & Exper. Therap.*, 33 (1928), 237) saw increases in pupil diameter varying from 0.8 mm. to 3.3 mm. in 10 individuals. This illustrates the variability of the response in man.

Three methods of biologic assay of ephedrine have been suggested, all of which are designed to overcome the interfering effect of tachyphylaxis.

Feng and Read (JOUR. A. PH. A., 16 (1927), 1034) made use of the pressor action, taking advantage of the small amount of tachyphylaxis induced by small doses given at long intervals. They gave intravenously 1 mg. per dog at hourly intervals, but apparently did little work with the method and report only one test. A dog weighing 5.2 kilos anesthetized with luminal was given at hourly intervals, 1 mg., 1 mg., 0.5 mg. and 2 mg. They do not state the pressor responses but give a reduced tracing showing the following rises on the reproduced tracing; 5 mm., 5 mm., $2^1/_2$ mm. and 7 mm.

Rowe (JOUR. A. PH. A., 16 (1927), 912) had previously studied the quantitative relationship between the pressor actions of epinephrine and ephedrine, and between the effects of the latter as given by intravenous injection and by mouth. His figures are, however, disturbed by the fact that he apparently did not recognize either the decreased action of ephedrine on repeated injection, or the increased action of epinephrine after ephedrine. In some cases he injected one first, in other cases the other, and he unites all results in a table showing a relationship varying from 1:100 to 1:1000.

Pittenger (JOUR. A. PH. A., 17 (1928), 634) checked Feng and Read's method on ten dogs and found that injection of 1 mg. caused enough tachyphylaxis to render the method untrustworthy. He suggests, as already noted, use of the mydriatic effect but reports no work with it.

To avoid the discrepant results due to individual differences, Chen (*J. Pharmacol. & Exper. Therap.*, 33 (1928), 237) suggested that each dog be "standardized" by determining its sensitiveness to epinephrine, and that the pressor effect of a single dose of ephedrine be compared to the pressor effects of a series of graded doses of epinephrine given previously. The strength of a given sample of ephedrine is thus expressed in terms of its relation to epinephrine. He believed that as epinephrine and ephedrine produced their pressor effects by a somewhat similar mechanism, animals should vary in their response to each of them to the same extent.

Our laboratories have been making an extensive study of certain compounds related to ephedrine and it was necessary during one phase of our work on one of them, phenylpropanolamine, briefly known as propadrin, to develop a method of biologic assay which would give quantitative results within 10 or 20 per cent.

We tried four methods: 1. The mydriatic action on the isolated frog's eye. 2. The action on the isolated uterus of the guinea pig. 3. The method used by Chen, modified by employing dogs anesthetized by chloretone, instead of pithed cats. 4. The method of Feng and Read, using similarly anesthetized dogs.

1. MYDRIATIC ACTION.

We can dismiss this method in a few words, by saying that although mydriasis was caused by all dilutions of propadrin down to 1:8000 (the weakest used), there was no relation between the strength of the solution and the degree of mydriasis. It was found that solutions of 1:8000 might cause more dilatation than 1:4000, and these more than 1:2000; even when the comparison was made between the two eyes of the same frog. Tests were conducted on 8 pairs of eyes, and the results were so unpromising that further work did not seem justified.

2. ACTION ON THE ISOLATED UTERUS.

Chen (*J. Pharmacol. & Exper. Therap.*, 33 (1928), 237) states that the action of ephedrine on the isolated uterus of the guinea pig does not lessen with repeated applications; on the other hand, Curtis (*J. Pharmacol. & Exper. Therap.*, 35 (1929), 333) found that there is lessened response in both the cat and guinea pig uterus, and this agrees with our own experience, with propadrin on the organ of the guinea pig. Figure 1, illustrating the response of the isolated uterus to re-

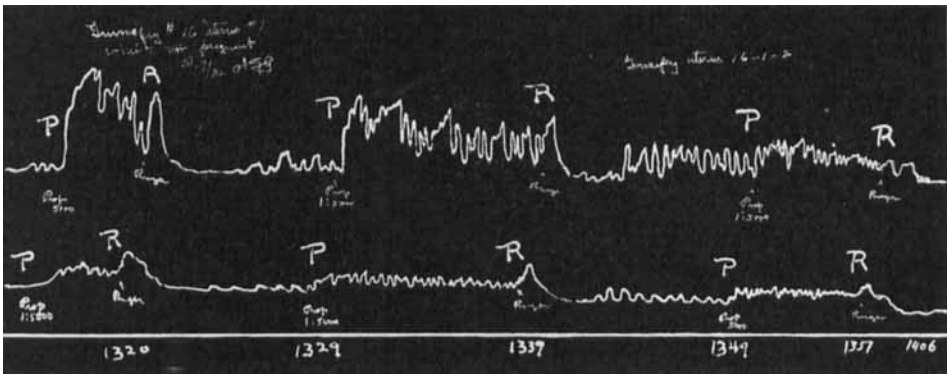

Fig. 1.—Isolated adult Guinea Pig uterus. Effect of repeated doses of same concentration of propadrin on two strips from the same uterus. At "P" propadrin 1:5000 wasadded to the bath. At "R" it was replaced by Ringer solution.

peated applications of the same concentration of propadrin, shows both the lessening response and the irregularity of the reduction. The method does not seem promising for quantitative studies.

3. PRESSOR RELATION TO EPINEPHRINE.

In testing out the method proposed by Chen we found that different dogs vary markedly in their response to ephedrine and propadrin. Thus, in a series of 40 dogs given doses of the latter varying from 55 to 1600 mg. per kilo, no quantitative relationship was found between dose and effect. For example, the 1600 mg. dose caused a rise of only 30 mm. of mercury, while another dog given only 390 mg. per kilo showed a rise of 110 mm. Five doses of 570 to 610 mg. per kilo, given to 5 different dogs, caused rises of, respectively, 20, 26, 32, 51 and 64 mm. The relation was no more exact by calculating the rise in terms of per cent of the original blood pressure. The same discrepancy between dose and effect, due to

variations in responsiveness in individual animals has been noted by other workers both with epinephrine and with ephedrine.

In the tests made on atropinized dogs, anesthetized with chloretone and morphine, we found a relationship of epinephrine to ephedrine varying from 1:120 to 1:1100 in 12 dogs, with an average of 1:495. Using 42 dogs in the same test with propadrin, the relation varied from 1:125 to 1:1000, the average being 1:495, the same as for ephedrine.

Our results would thus give ephedrine a much lower activity as compared to epinephrine than that found by Chen on pithed cats, but much higher than recently reported by Hasama (*Arch. exptl. Path. Ther.*, 153 (1930), 161) who found a relation of 1:3200 in rabbits.

If we take the average rise of pressure caused by a given dose of epinephrine and divide the dogs into two groups according to whether they were above or below average susceptibility and do the same with the response to propadrin, we find that of 40 dogs, 29 varied in the same direction, that is, they were above or below average susceptibility to both drugs, while in 11 the susceptibility to one drug was above average, that to the other below. Further evidence in this same direction, showing a lack of relation in susceptibility to the two drugs, is given by the fact that if we select the 7 dogs which were most susceptible to epinephrine, as shown by their giving a rise of pressure of 90 mm. or more from a dose of 20 mg. of epinephrine, the average relationship of propadrin to epinephrine was 1:738 as compared to 1:495 for the whole group. This shows that undue susceptibility to epinephrine does not extend to propadrin. Similar figures are found with ephedrine.

In some animals which seemed to be unduly deeply anesthetized by the chloretone, and in which the original blood pressure was very low, the response to ephedrine or to propadrin seemed to be more reduced than that to epinephrine. This does not account for the lower relative effect of ephedrine in our anesthetized animals as compared to Chen's pithed animals, as relationships as low as 1:1000 were found in a small series of pithed dogs.

The wide discrepancy found by Chen in a series of only 6 animals (relations varying from 1:133 to 1:294) shows that with pithed animals also, a very large number is required to obtain accurate quantitative results with any one sample.

4. EFFECT OF REPEATED DOSES ON BLOOD PRESSURE.

Not having obtained satisfactory results by the method just described, we turned to the earlier method of Feng and Read. The results were more satisfactory, but still leave much to be desired. By the method described below it was possible to obtain results within 20 or even within 10 per cent with only one or two dogs on each test. The method is as follows:

Anesthetized dogs are given repeated doses of ephedrine or propadrin intravenously, at intervals of an hour. Each dose contains 1 mg. of a standard solution or an approximately equal amount of the solution under test. The dose is not adjusted to the weight of the dog, as we found no relation between this and the response to a fixed dose. Large dogs are almost if not quite as likely to give a considerable rise as are small ones. The dog is first given 1 mg. of the standard preparation, and the rise measured. At the end of an hour it is given the solution

under test, and at the end of another hour the standard once more in the same dose. In this way we continue at hourly intervals until, preferably, four doses of each have been given. The averages of all the doses of each sample are then taken. If there be a discrepancy of more than 20 per cent between them, it can be fairly safely concluded that this represents a real difference in the potency of the solutions. In some dogs the rise from the first injection is unduly large, and in such cases this rise is excluded in casting the averages. Quite satisfactory results may be obtained if only half an hour is allowed between doses, but the hourly interval is probably best.

A few illustrative protocols will serve to show the kind of results obtained by this method.

Dog No. 168. Weight, 7.3 kilos. Comparison of two 1 per cent solutions of the same lot of propadrin, marked, respectively, No. 3 and No. 18.

Time.	Injection No.	Dose, Mg.	Rise, Mm.
10.55	18	1	22
11.55	3	1	12
13.00	3	1	12
14.00	18	1	10
15.00	18	2	14
16.00	3	2	14

The first rise was unduly high and is, therefore, omitted in calculating averages.

Average of other injections of No. 18—13 mm.

Average of all injections of No. 3—12 mm.

The result is within 10 per cent, the solutions are, therefore, of approximately the same activity.

Dog. No. 183. Weight, 8.15 kilos. Repeated injections of same solution of propadrin.

Time.	Injection No.	Dose, Mg.	Rise, Mm.
10.10	3	1	24
10.40	3	1	18
11.40	3	1	16
12.40	3	1	14
14.05	3	1	16
14.55	3	1	16
15.45	3	1	18
16.15	3	1	14
16.45	3	1	14

Disregarding the first aberrant response, we take every alternate rise and cast an average. The sum of the 2nd, 4th, 6th and 8th is 64 mm.; the sum of the 3rd, 5th, 7th and 9th is 62 mm. The averages being 16 mm. and $15^1/_2$ mm., a variation of only 3 per cent.

Dog No. 170. Weight, 9.1 kilos. Morphine, chloretone, atropine anesthesia. Comparison of two solutions of standard propadrin. A contains 10 mg. per cc.; B, 8 mg. per cc.

Time.	Injection.	Dose, Cc.	Rise, Mm.
11.40	B	0.1	6
12.10	A	0.1	12
12.50	B	0.1	8
13.20	A	0.1	10
13.50	B	0.1	10
14.20	A	0.1	14
15.48	B	0.1	14
16.21	A	0.1	12

Average rise from A 13½ mm.; from B, 10 mm., showing a distinct variation in activity in favor of A.

Dog No. 172. Weight, 5.5 kilos. Morphine, chloretone, atropine anesthesia. Comparison of three solutions of standard propadrin. A contains 8 mg. per cc. B contains 6.7 mg. per cc. C contains 5 mg. per cc.

Time.	Injection.	Dose, Cc.	Rise, Mm.
11.00	A	0.1	14
11.40	B	0.1	12
12.27	C	0.1	6
14.30	A	0.2	10
15.00	B	0.2	8
15.30	B	0.2	10
16.00	C	0.2	6
16.30	B	0.2	12
16.55	A	0.2	12

Average rise from 1 injection of 0.1 cc. and 2 of 0.2 cc. A, 12 mm.; B, 10²/₃ mm.; C, 7⅓ mm. Showing that A is stronger than B and this than C.

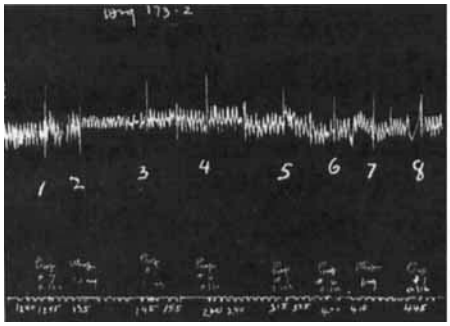

Fig. 2.—Dog 175. See Protocol.—Effect on blood pressure of small repeated doses of propadrin. Intravenous injection given as follows: 1, 12.45 propadrin No. 7, 0.1 cc. 2, 1.35 atropine. 3, 1.45 propadrin, No. 1, 0.1 cc. 4, 2.30 propadrin No. 6, 0.1 cc. 5, 3.15 propadrin No. 1, 0.1 cc. 6, 4.00 propadrin No. 6, 0.1 cc. 7, 4.10 atropine. 8, 4.45 propadrin No. 1, 0.1 cc.

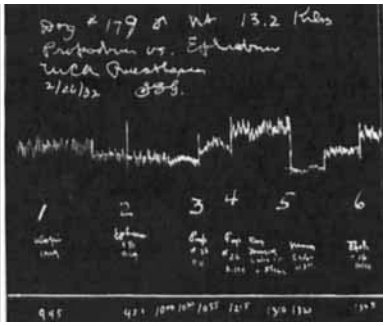

Fig. 3.—Dog 179. See Protocol.—Effect on blood pressure of small alternate doses of ephedrine and propadrin. Intravenous injections given as follows: 1, 9.40, atropine. 2, 9.50, ephedrine, 1%, 0.1 cc. 3, 10.55, propadrin 1%, 0.1 cc. 4, 12.45 propadrin, 1%, 0.1 cc. 5, Dog moving. Gave ether. 6, 1.45 ephedrine, 1%, 0.1 cc.

These few protocols will show the general character of the results obtained by this method, and that, even with only one or two animals it is accurate to within 20 or perhaps even 10 per cent. It is necessary to give repeated injections of each sample, and to take the average of the rises obtained, as there is always some variation in the rise from the same dose. The results are probably better if an hour is allowed to elapse between injections, although quite good results may be obtained with an interval of half an hour. If the rise obtained from 0.1 cc. of a 1 per cent solution is not more than 15 mm., doses of 0.2 cc. may be given, and will give a better contrast.

Quantitative studies, both by determining the pressor relation to epinephrine and by comparing the repeated effect of small doses, were made to determine the

stability of propadrin and ephedrine in 1 per cent solution in distilled water. Certain of these samples were subjected to influences, such as passing oxygen through the solution for 2 hours, incubation at 37° C. for 7 days, autoclaving for 50 minutes at 120° C. Of the lots subjected to each of these treatments, some samples contained possible protective agents including glucose, lactose, a trace of the free base and an antiseptic, S. T. 37 solution containing hexylresorcinol. Neither ephedrine nor propadrin solutions showed any loss of activity when subjected to the above treatments. The presence of protective agents was found to be unnecessary and the solutions associated with hexyl-resorcinol were not affected either by exposure to high temperatures or to oxygen.

The following protocol is typical of the results obtained. Solution No. 1 had been kept in the refrigerator: No. 7 was heated at 120° C. for 50 minutes; and No. 6 was incubated at 37° C. with hexyl-resorcinol (S. T. 37) solution.

Dog No. 173. Weight, 10.5 kilos. Morphine, chloretone, atropine anesthesia. Comparison of standard, sterilized and incubated samples of propadrin (Fig. 2).

Time.	Injection Propadrin Solution.	Dose, Cc.	Rise, Mm.
9.53	1	0.1	50
10.15	7	0.1	40
10.45	1	0.1	32
11.15	7	0.1	24
11.45	1	0.1	14
12.45	7	0.1	34
13.45	1	0.1	26
14.30	6	0.1	32
15.15	1	0.1	24
16.00	6	0.1	24

Although there is some lessening of effect from each of the first 5 injections given at intervals of only half an hour, this is adjusted by giving alternate injections and recovery of effect is seen when the interval is lengthened to one hour. Averaging the effects of each sample we find: No. 1, average 29 mm. No. 7, average $32^2/_3$ mm. No. 6, average 28 mm.

These results show that neither No. 6 nor No. 7 is appreciably weakened by the treatment to which it has been exposed.

It has already been mentioned that propadrin and ephedrine showed the same relative pressor action when compared to epinephrine. This finding was confirmed by two tests in which 1 mg. per dog of each were given alternately and the resulting rises of pressure compared. The protocols follow:

Dog No. 179. Weight, 13.2 kilos. Morphine, chloretone, atropine anesthesia. Comparison of 1 per cent solutions of ephedrine and of propadrin (Fig. 3).

Time.	Injection, %.	Dose, Cc.	Rise, Mm.
9.52	Ephedrine 1	0.1	24
10.55	Propadrin 1	0.1	22
12.45	Propadrin 1	0.1	24
13.45	Ephedrine 1	0.1	22

Average rise from ephedrine, 23 mm. From propadrin, 23 mm.

Dog No. 177. Weight, 11.8 kilos. Morphine, chloretone, atropine anesthesia. Comparison of 1 per cent solutions of ephedrine and of propadrin.

Time.	Injection, %.	Dose, Cc.	Rise, Mm.
9.55	Propadrin 1	0.1	22
10.45	Ephedrine 1	0.1	18
11.30	Propadrin 1	0.1	12
12.30	Ephedrine 1	0.1	12
13.30	Propadrin 1	0.1	12
14.50	Ephedrine 1	0.1	12

After the first two injections the effects agree exactly. Even including them, the ephedrine effects average 14 mm., the propadrin 15 1/3, the results being within 10 per cent.

These protocols illustrate the close agreement in quantitative action and stability of propadrin and ephedrine.

SUMMARY AND CONCLUSIONS.

1. Of the four methods used for the quantitative physiologic assay of propadrin and ephedrine, namely, the effect on the pupil, the effect on the isolated uterus, the comparison of the pressor action with that of epinephrine and the comparison of the pressor action with that of a standard solution of the same drug, only the last was found to be suitable.

2. By the method described, assays can be made on one or two dogs with an accuracy of 20 or even of 10 per cent.

3. Propadrin is of approximately the same activity as ephedrine and is equally stable in solution.

A STUDY OF SENECIO RIDDELLII.*

BY F. S. BUKEY AND R. W. CUNNINGHAM.[1]

PART 1.

INTRODUCTION.

A number of Senecio such as *S. integerrimus, S. riddellii, S. vulgaris, S. ilicifolius, S. burchellii, S. latifolius, S. jacobœa, S. aureus, S. præcox, S. canicidia, S. albicaulis, S. grayanus, S. cervariæfolius* and many others, have been found in many parts of the world. *A ureus* is official in the N. F. V and has enjoyed some use as a drug. It was employed by the Indians as a vulnerary and later was used as an emmenagogue. Some of the above-mentioned species have been used as purgatives, emetics and internal hemostatics, although of doubtful value in most cases.

It has been found that some of these species are poisonous to animals. Debrierri (1) reports the poisonous effects of canicidia on dogs. Gilruth (2), (3), (4), (5), (6), (7), (8) proved the toxicity of *S. jacobœa* in producing cirrhosis of the liver (Winton disease) in horses of New Zealand. Chase (9), Robertson (10), Dixon (11) and Theiler (12), (13) succeeded in proving *S. latifolius* and *S. burchellii* to be the cause of hepatic cirrhosis or Malteno disease of South Africa. Stockman (14), Thompson (15), Craig and Kehoe (16), Stanley (17), Knowles (18), Leychon (19), Reeks (20) and Rutter (21) all report cases of poisoning by *S. jacobœa* among the

* Scientific Section, A. PH. A., Toronto meeting, 1932.

[1] The authors wish to express their thanks to Dr. L. Van Es of the department of Animal Pathology, University of Nebraska, for his coöperation and assistance in this study.