The Nervous System of the American Leopard Frog, Rana Pipiens, Compared with that of the European Frogs, Rana Esculenta and Rana Temporaria (Fusca)

By HENRY H. DONALDSON

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THE NERVOUS SYSTEM OF THE AMERICAN LEOPARD FROG, RANA PIPIENS, COMPARED WITH THAT OF THE EUROPEAN FROGS, RANA ESCULENTA AND RANA TEMPORARIA (FUSCA).

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WITH SIX FIGURES.

With the advances which are being made in the correlation of function and structure, the need is felt on many sides for a more detailed, accurate and quantitative determination of the anatomical, physiological and chemical differences between closely related species, as well as between the same species from different localities.

The general notion of a physiological and chemical criterion for species has been discussed by DE VARIGNY ('99) and, although this is not the place to review the literature touching this topic, it is nevertheless appropriate to name CAMERANO's paper ('00) on the variation of the toad, which, among his many important contributions in this general field, is the one most closely related to the following investigation. Moreover, KELLICOTT's recent study of correlation and variation in internal and external characters in the common toad ('07) emphasizes relations which have a direct bearing on the interpretation of my own results.

In 1898 I made a study of the weight of the brain and of the spinal cord in the bull-frog R. catesbiana (DONALDSON '98). Two years later, in collaboration with Dr. D. M. SCHOEMAKER, a similar series of observations on the leopard frog, R. pipiens,[1] was published (DONALDSON and SCHOEMAKER '00).

In 1902, utilizing the data in both of these investigations, I was able to show that the weight of the central nervous system in both of these species could be calculated by a formula based on the body weight and on the total length of the frog (DONALDSON '02).

For comparison with these results the observations of FUBINI ('81) on the European frogs were alone available.

An examination of FUBINI's tables, which are discussed in part in my paper of 1898, referred to above, showed that his findings were so irregular and so different from my own, that it was fair to conclude that he had not worked with sufficient care.

In order to test this conclusion, I obtained in the spring of 1898, through the courtesy of the Zoölogical Institute at Zurich, Switzerland, a series both of R. esculenta and R. temporaria (fusca), all the specimens having been fixed in formalin by a uniform method. A comparison of these specimens with the fresh R. pipiens on the one hand, and on the other with R. pipiens fixed by the same method, indicated that the central nervous system in R. pipiens was heavier than in the European species, and at the same time did not support any of the peculiar findings of FUBINI, such as the relatively great weight of the spinal cord. Nevertheless, limitations in the range in size of the Zurich series and the possibility that the European and American species were differently affected by the fixation treatment, led me to delay publication on this point until fresh material could be examined. The opportunity to do this came in the summer of 1904. In July of that year, through the courtesy of Professor SHERRINGTON, I was able to examine a series of R. temporaria (fusca) in the physiological laboratory of University College at Liverpool, England; and in August, through the courtesy of Professor GAULE, a corresponding series of R. esculenta was examined in the Physiological Institute of the University at Zurich, Switzerland.

In order to eliminate as far as possible, the influence of *season* on this comparison, Dr. HATAI examined for me, also in August, a series of R. pipiens in the Neurological laboratory of the Uni-

[1] In previous papers on the leopard frog, published from my laboratory, this species has been designated as Rana virescens brachycephala, Cope. It now appears that this name is not correct, and that the species in question should be designated Rana pipiens (Schreber) as given above (DONALDSON, *Science*, vol. 26, p. 655, 1907.)

versity of Chicago. It is the results of these three series of observations which are now to be compared.

As the foregoing shows, this investigation was undertaken primarily to test the correctness of FUBINI's observations. It has resulted however in bringing to light several differences between the nervous systems of the species compared, and these differences seem worth recording. At the same time, FUBINI's observations have been found untrustworthy. This, however, is a matter of small importance, and the brief discussion of FUBINI's work will be deferred to an appendix.

Before presenting the data on the nervous system, it will be desirable to record some of the characters in which these three species of frogs closely resemble one another. The resemblances important for our present purpose are enumerated below:

(1) In external appearance and shape; color markings excepted.

(2) In the range in body weight (the heaviest specimens are always females).

(3) In the ratio obtained by dividing the body weight by the total length, that is, the average amount of body weight for each running millimeter of total length.

It will be necessary to interrupt the enumeration for a moment in order to elaborate this point (3).

In the full tables are given the body weight and the total length of each individual examined. In the condensed Table 10, these same data are arranged to give the averages for *groups of three.* Thus for each species in the latter table there are four entries, and in each entry both the average body weight and the average total length are given. If the former be divided by the latter

$$\frac{\text{Body weight}}{\text{Total length}}$$

we obtain a number which represents the average amount of weight for each running millimeter. Since the increase in body weight is more rapid than the increase in total length, this value of course changes with the absolute weight of the frog, increasing as the frog becomes absolutely heavier. The values thus obtained are given in Table 1.

These values are better understood when thrown into a curve, as in Chart 1.

It appears from the chart that the curves are nearly parallel,

TABLE 1.

	Body Weight in Grams.	Body Weight per Millimeter in Grams.
R. pipiens	14.9	.102
	23.2	.135
	30.8	.168
	43.2	.218
R. esculenta	15.9	.114
	22.0	.134
	35.0	.199
	40.2	.208
R. temporaria	15.9	.107
	23.1	.137
	28.0	.162
	31.3	.177

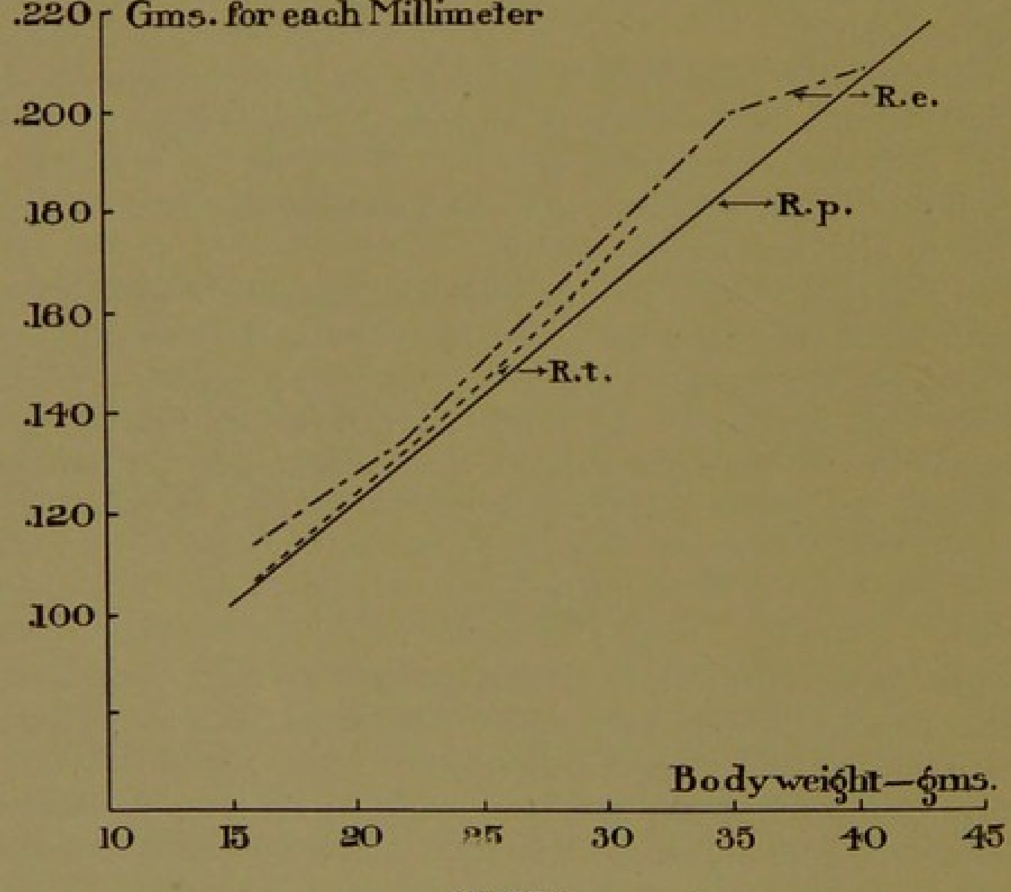

CHART 1.

Showing the average amount of body weight for each running millimeter of total length.
R. p. = Rana pipiens.
R. e. = Rana esculenta.
R. t. = Rana temporaria.

and that on the average, the lower figures differ only 4.1 per cent (R. pipiens) and 2.3 per cent (R. temporaria) respectively, from the highest figures, given by R. esculenta. The relation of body weight to total length is therefore nearly the same in all three species.

(4) In the fraction of the total length represented by the combined lengths of the leg bones.

Table 2 gives these figures in their final form.

TABLE 2.

Percentage of the total length represented by the combined lengths of the leg bones.

	No. of Specimens.	
R. pipiens	12	68.7%
R. esculenta	5	70.7%
R. temporaria	6	69.4%

The percentages in the foregoing tables were obtained as follows: That for R. pipiens from an average of twelve records on individuals ranging from 14.85 to 42.54 grams in body weight (DONALDSON and SCHOEMAKER, '00, Table VII); that for R. esculenta from five specimens of the Zurich series of 1898, having a body weight of 12.3–20.4 grams; and that for R. temporaria (fusca) from six specimens of the same series ranging in body weight from 17.9–34.7 grams.

Table 2 serves to show that in this character the three species are nearly alike.

(5) In the proportional lengths of the several leg bones.

TABLE 3.

	No. of Specimens.	Femur.	Tibia.	Foot (Tarsus and Pes).
R. pipiens	12	25.5%	29.3%	45.2%
R. esculenta	5	26.3%	28.2%	45.5%
R. temporaria	6	26.1%	28.7%	45.2%

The figures in Table 3 are based on the same data as were used for Table 2. For comparison in the case of R. esculenta however, we have in addition, the measurements from BOULENGER ('97). These are taken both from his tables and from measurements made on the bones as represented in his plates.

The data from BOULENGER give the following:

TABLE 4.

R. ESCULENTA.	NO. OF SPECIMENS.	FEMUR.	TIBIA.	FOOT (TARSUS AND PES).
Variety rabibunda	1 M.+1 F.	26.7%	29.2%	44.1%
Variety rabibunda	1 F.*	27.7%	28.9%	43.4%
Variety typica	1 M.+1 F.	26.8%	27.3%	45.9%
Variety lessonæ	1 M.+1 F.	25.9%	26.5%	47.6%

* Measured from Boulenger's Fig. 101, p. 280.

In my Zurich series the individual measurements correspond to those for the varieties *rabibunda* and *typica* as determined by BOULENGER. The average for these from the above table (4) is:

AVERAGE VALUES FOR VARIETIES RABIBUNDA AND TYPICA.

Femur	27.0
Tibia	28.3
Foot	44.7

And these values are close to those given for R. esculenta in Table 3.

For comparison in the case of R. temporaria, an average of two determinations, one male, one female, by BOULENGER is available. These give

Femur	25.6%
Tibia	28.4%
Foot	46.0%

which is in fair agreement with the values given in Table 3.

(6) In the relative length of the entire central nervous system (that is, the length of the brain plus the length of spinal cord), in relation to the total length of the frog.

This relation is of course not a constant one, because the total length of the frog increases more rapidly than the length of the entire nervous system. To make the comparisons, therefore, the percentages must be recorded in relation to the total length found for each individual or group. The data used in this determination were the following:

Ten (10) specimens of R. pipiens ranging in total length from 124 mm. to 185 mm. inclusive. The data being taken from the research of DONALDSON and SCHOEMAKER ('00). These cases are averaged in groups of five.

Twelve (12) specimens of R. pipiens, these being the same as are given in Table 7 and averaged in groups of four (observations by Dr. HATAI). Both of the foregoing series of measurements were made on the fresh specimens.

Ten specimens of R. pipiens, after fixation in formalin, averaged in groups of five, and ranging in total length from 128–174 mm. This was the series used to control the measurements on the European frogs received from Zurich in 1898.

From the Zurich series of 1898, there were taken one group of five esculenta, ranging in total length, after fixation, from 128–170 mm., and also a series of fifteen R. temporaria, averaged in groups of five, and ranging in total length, after fixation, from 151 to 179 mm.

It seems fairest to tabulate the fresh, separately from the fixed material, so the first lot, entirely R. pipiens, is given in Table 5.

TABLE 5.

R. pipiens. Percentage values of length of the entire central nervous system, the total length of the frog being taken as the standard. Measurements on fresh specimens.

Averages of	Total Length mm.	Per cent Value of the Length of Entire Central Nervous System.
4	150	17.2
5	153	17.8
5	175	17.2
4	177	16.2
4	196	16.3

TABLE 6.

Showing the same relation as Table 5. All measurements on material fixed in formalin.

Species.	Average of	Total Length mm.	Per Cent Value of the Length of the Entire Central Nervous System.
R. pipiens	5	133	18.2
R. esculenta	5	145	17.9
R. temporaria	5	158	16.9
R. temporaria	5	166	16.2
R. pipiens	5	167	16.4
R. temporaria	5	173	16.0

Table 5 shows that the value in question ranges in the fresh specimens from 17.8 per cent to 16.2 per cent, and also tends to diminish as the total length of the frog increases. The same relations are shown in Table 6, in which all three species are represented, and these form as satisfactory a series as is given in Table 5. We therefore conclude that in this character—the relative length of the entire central nervous system—the three species resemble one another closely.

It should be pointed out here that it follows from this that the smaller weight of nervous system which we find in the European forms (see below) must be associated with a diminution of one or both the transverse diameters, since the foregoing shows that it is not associated with variations in total length.

(9) In the arrangement of the main branches of the crural and sciatic nerves (DUNN '00 and '02). In the papers to which reference is here made, this point is fully discussed.

In view of the fact that the several species are similar in the foregoing characters, we might expect a high degree of similarity in the weight and structural relations of the central nervous system. Such however is not the case, and we turn therefore to a statement of the differences which have been observed.

The technique of weighing, measuring and dissecting, was uniform for the three species. This has already been described (DONALDSON, '98, DONALDSON and SCHOEMAKER, '00).

It may, however, be well to repeat here that the body weight was taken in a closed box; the weight of the contained ova being deducted from the body weight of the unopened specimen, in the case of the females. Also in both sexes correction was made for the stomach contents.

In taking the total length, the frog was suspended by the lower jaw, and the distance between the tip of the nose and the longest toe, the legs being fully extended, was measured with vernier calipers. The central nervous system was removed immediately after death, and the brain separated from the spinal cord by a section at the level of the tip of the calamus scriptorius. Both brain and cord were separated from their nerves by severing the latter at the points of their attachments to the central structures. To obtain the percentage of water, the material was dried for several days until it maintained a constant weight. For this a water bath ranging from 85° to 95° C. was used.

Although this is probably not the best method, it was uniformly applied in the case of all three series, so that the results are at least comparable, though the absolute values for the percentage of water may be open to question, until it has been shown that this material dried in vacuo, gives similar results.

Material examined.—The specimens of R. pipiens were 12 in number (10 males and 2 females) ranging in body weight from 11.6–47 grams. They were taken in the neighborhood of Chicago in the month of August, and examined between the twenty-third and thirty-first of August. For the data which are presented in Table 7 I am indebted to Dr. S. HATAI.

TABLE 7.

Data on R. pipiens.

Body Weight in grms.	Sex.	Total Length in mm.	Weight in Grams of			Ratio of Brain Weight to Cord Weight.	Percentage of Water.	
			C. N. S	Brain.	Sp. C.		Brain.	Sp. C.
11.6	M.	130	.0918	.0666	.0252	2.64	84.4	79.4
16.0	M.	150	.1148	.0796	.0352	2.26	85.2	80.7
17.0	F.	159	.1054	.0714	.0340	2.10	84.0	80.6
20.8	M.	170	.1232	.0844	.0388	2.17	85.2	81.6
22.5	M.	162	.1165	.0807	.0358	2.25	84.5	80.4
26.4	M.	180	.1372	.0946	.0426	2.22	84.4	78.4
27.6	F.	179	.1416	.1014	.0402	2.52	84.8	80.1
30.6	M.	180	.1454	.0998	.0456	2.18	84.6	79.8
34.2	M.	190	.1518	.1056	.0462	2.28	85.6	81.6
41.8	M.	197	.1652	.1146	.0506	2.26	86.9	82.2
43.9	M.	200	.1708	.1210	.0498	2.42	85.8	80.7
47.0	M.	198	.1664	.1140	.0524	2.17	84.4	80.5

The specimens of R. esculenta were eleven in number (3 males and (8 females) ranging in body weight from 12.4–45.03 grams. They were taken near Zurich on July 31, and were examined between August 1 and 5. The data are given in Table 8.

The specimens of Rana temporaria (fusca) were twelve in number (8 males and 4 females) ranging in body weights from 14.05–32.81 grams. They were taken near Liverpool shortly before July 11, and were examined July 11 and 12. The data are given in Table 9.

In all the foregoing series there is considerable individual variation in the characters observed, and so for the purposes of comparison, the complete tables have been condensed by taking the averages for each three successive individuals, thus giving four entries in each of the condensed tables.

The only exception to this statement is in the case of R. esculenta, with but 11 records, and there the third entry in the con-

TABLE 8.

Data on R. esculenta.

Body Weight in grms.	Sex	Total Length in mm.	Weight in Grams of			Ratio of Brain Weight to Cord Weight.	Percentage of Water	
			C. N. S.	Brain.	Sp. C.		Brain.	Sp. C.
12.40	F.	131	.0818	.0577	.0241	2.39	84.2	78.4
16.75	F.	144	.0926	.0634	.0292	2.17	83.4	79.1
18.43	F.	144	.0928	.0650	.0278	2.34	83.2	78.2
20.00	F.	161	.1103	.0756	.0347	2.17	82.5	79.2
22.00	F.	164	.1107	.0769	.0338	2.27	84.0	79.0
24.10	M.	167	.1217	.0841	.0376	2.23	83.4	78.4
33.85	M.	175	.1327	.0895	.0432	2.07	83.2	78.2
36.30	M.	177	.1478	.1004	.0474	2.11	83.4	78.6
37.56	F.	188	.1490	.0993	.0497	1.99	82.9	78.8
37.96	F.	194	.1427	.0953	.0474	2.01	82.8	77.8
45.03	F.	196	.1578	.1078	.0500	2.15	83.9	78.4

TABLE 9.

Data on R. temporaria.

Body Weight in grms.	Sex	Total Length in mm.	Weight in Grams of			Ratio of Brain Weight to Cord Weight.	Percentage of Water.	
			C. N. S.	Brain.	Sp. C.		Brain.	Sp. C.
14.05	F.	144	.0881	.0596	.0285	2.09	82.3	78.2
16.10	F.	151	.0991	.0690	.0301	2.22	82.7	79.0
17.65	M.	154	.0916	.0618	.0298	2.07	83.0	78.5
21.75	M.	171	.1045	.0671	.0374	1.79	82.8	78.2
23.45	M.	162	.0947	.0628	.0319	1.96	82.1	77.0
24.17	F.	173	.1333	.0864	.0469	1.84	81.9	76.5
27.05	M.	173	.1298	.0874	.0424	2.06	82.4	77.1
28.15	M.	168	.1018	.0687	.0331	2.07	82.5	76.7
28.95	M.	174	.1324	.0813	.0511	1.59	81.3	76.8
28.95	M.	178	.1485	.0928	.0557	1.66	81.3	76.8
32.15	M.	173	.1321	.0890	.0431	2.06	80.9	78.6
32.81	F.	178	.1161	.0766	.0396	1.93	82.7	78.0

densed tables is based on only two individuals, numbers 7 and 8 in the series. The condensed tables are given in connection with each character discussed. That for the weight of the entire central nervous system follows:

TABLE 10.

Weight of the central nervous system in grams, averages from groups of three.

	BODY WEIGHT	WEIGHT OF CENTRAL NERVOUS SYSTEM.	TOTAL LENGTHS IN MM.
R. pipiens	14.9	.1040	146
	23.2	.1256	171
	30.8	.1463	183
	43.2	.1674	198
R. esculenta	15.9	.0890	139
	22.6	.1142	164
	35.0	.1402	176
	40.2	.1498	193
R. temporaria	15.9	.0929	149
	23.1	.1108	167
	28.0	.1213	173
	31.3	.1323	176

The data in the foregoing tables are also presented in Chart 2 and the relations are more easily discussed by reference to the chart, which shows R. pipiens to have the heaviest central nervous system, R. esculenta the next heaviest, and R. temporaria the lightest.

By measuring the differences between the several entries for the two European species, and the corresponding points on the curve for R. pipiens, which is taken as the standard, it appears that on the average, the central nervous system of R. esculenta weighs about 89 per cent, and that of R. temporaria 88 per cent, of that found in R. pipiens.

It is known that dry air and starvation (DONALDSON '98, DONALDSON and SCHOEMAKER '00) tend to reduce the weight of the living frog, and probably of the central nervous system, also that the weight of the latter is increased in frogs which are moribund, and have consequently taken up an excessive amount of water.

There is no reason to think however that the foregoing observations are seriously modified by any of these influences.

Moreover unpublished observations on R. pipiens, in my possession, indicate a variation in the weight of the central nervous system with season. Nevertheless from the middle of June to the middle of September, such variations as occur, are hardly significant, and the observations here used were made within fifty-one days (July 11 to August 31) and fall within the general

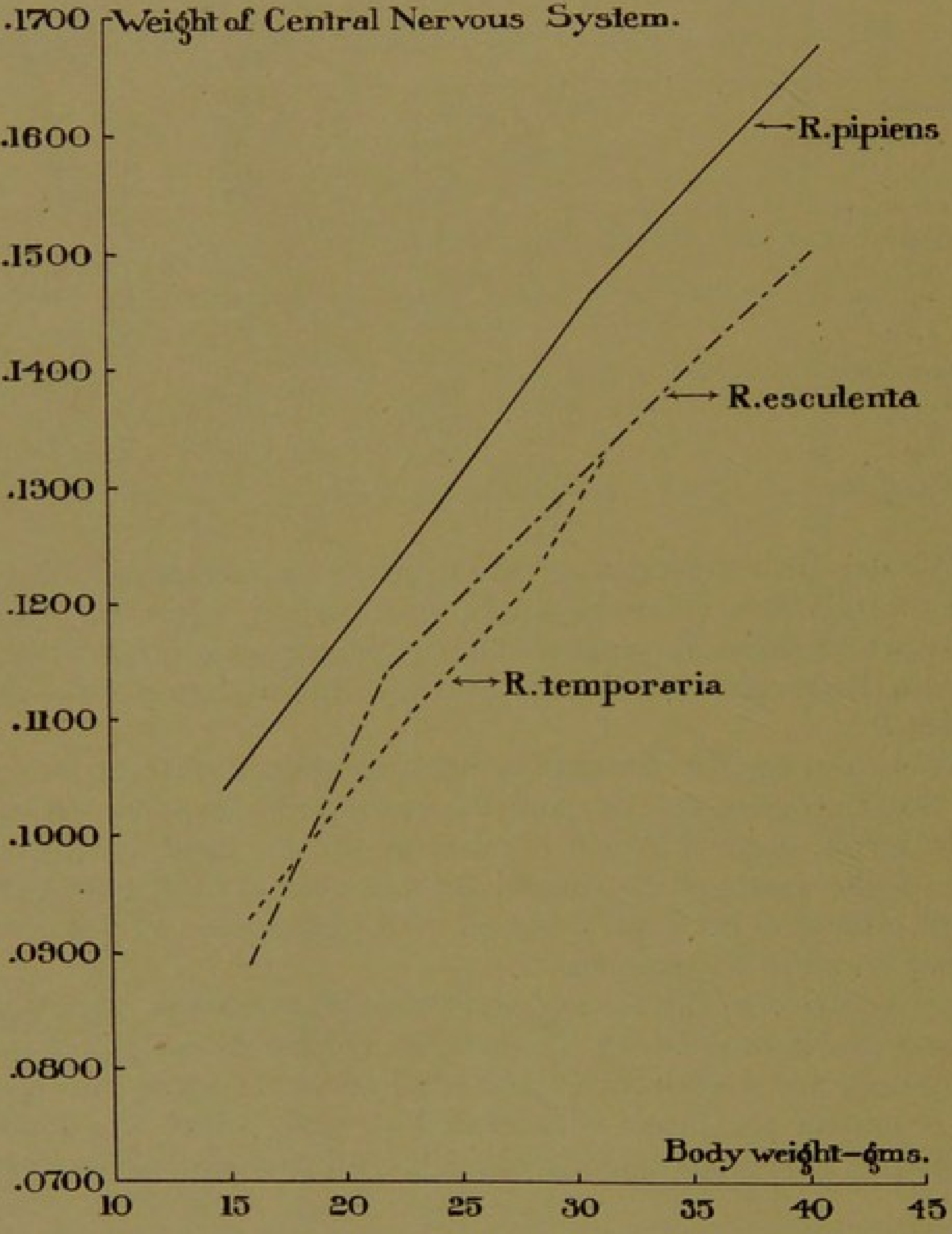

CHART 2.
Showing the weight of the entire central nervous system.

seasonal limits for constancy, as given above. This summer period is moreover the one during which the central nervous system shows its greatest weight. At the same time it is worthy of note that the results obtained on R. pipiens in August, 1904, correspond with the *lowest* weights found during August, 1901, in the case of the unpublished series.

In one sense this is perhaps fortunate, because it shows that the values here reported for R. pipiens are minimal, and if those for the European forms are also minimal, then the differences are approximately normal. If, on the other hand, the values for the European species are higher than the minimal, then the differences here given are less than they should be. In any case, and this is the main point, it follows that the differences here given are not exaggerated. I conclude therefore that the European species have a central nervous system which weighs from 11 per cent to 12 per cent less than that of R. pipiens.

As the Chart 2 shows, the curves for the weight of the central nervous system run nearly parallel, and as in a previous study (DONALDSON '02) R. pipiens has been found to conform to the formula for the determination of this weight, which is based on the body weight and total length of the frog, it follows that the European species would also conform to this same formula.

The formula contains a constant, C., which is different for each species, and which is modified by the general condition of any series. In the series of R. pipiens of 1902, the value of the constant C. was 28. In the present series of R. pipiens which, as has been noted, yields a low weight for the central nervous system, the value of C. is 26, and we should anticipate that it would be less for the two European species.

On making the calculations, I find the following values for C.

R. pipiens	C= 26
R. esculenta	C= 24
R. temporaria	C= 23

Our expectation then that the formula for the European species would have smaller values of C. is shown to be warranted.

On separating the weight of the brain from that of the spinal cord, and recording them separately, we have the relations given in Table 11.

Presenting these results in a form of a chart (Chart 3) it is seen that the brain weights for the several species follow the same order as that of the weight of the entire central nervous system, the superiority of R. pipiens being even more marked. The weights for the spinal cords however run much closer together.

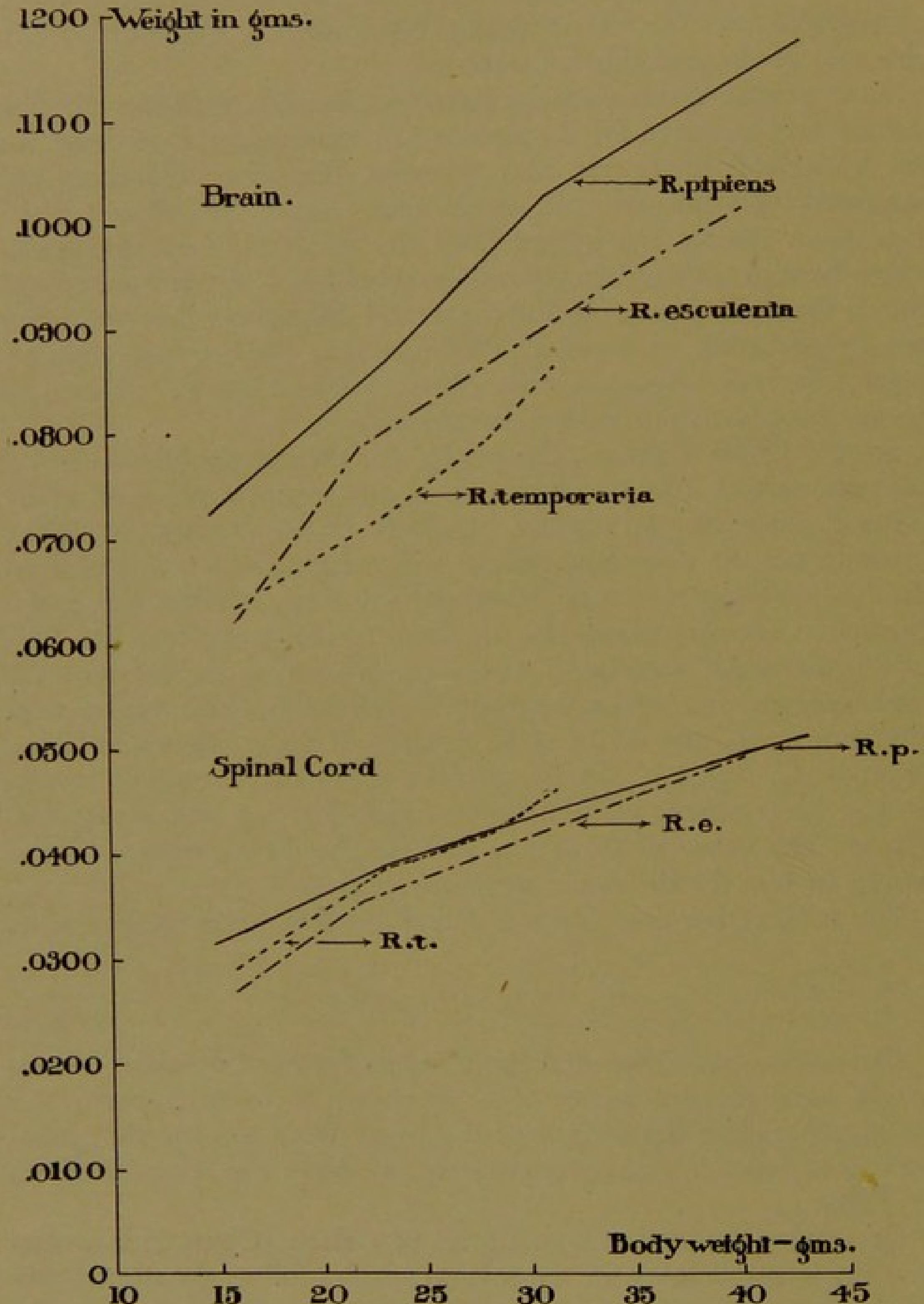

CHART 3.

Showing the weight of the brain and of the spinal cord.

R. pipiens has still the heaviest cord, but R. temporaria, with the lightest entire central nervous system, has a spinal cord nearly as heavy as that of R. pipiens, while the cord in Rana esculenta is distinctly lighter than in the other two species, having on the average 94 per cent of the weight of the cord in R. pipiens.

TABLE 11.
Weight of the brain and spinal cord in grams. Averages from groups of three.

	Body Weight.	Brain.	Spinal Cord.
R. pipiens	14.9	.0725	.0315
	23.2	.0866	.0390
	30.8	.1023	.0440
	43.2	.1165	.0509
R. esculenta	15.9	.0620	.0270
	22.0	.0788	.0354
	35.0	.0949	.0453
	40.2	.1008	.0490
R. temporaria	15.9	.0635	.0294
	23.1	.0721	.0387
	28.0	.0791	.0422
	31.3	.0862	.0461

To show the relative weight of the brain as compared with that of the cord in these three species, we may use the ratio obtained by dividing the brain weight by the cord weight. These ratios are given in the following table:

TABLE 12.
Ratios of the weight of the brain to the weight of the spinal cord. Averages from groups of three.

	Body Weight.	Ratio.
R. pipiens	14.9	2.33
	23.2	2.22
	30.8	2.32
	43.2	2.28
R. esculenta	15.9	2.29
	22.0	2.22
	35.0	2.09
	40.2	2.05
R. temporaria	15.9	2.15
	23.1	1.86
	28.0	1.87
	31.3	1.87

Putting these data in the form of curves in Chart 4, we see that the relative brain weights follow the order of the absolute weights of the entire nervous system and of the brain, the highest ratios being given by R. pipiens.

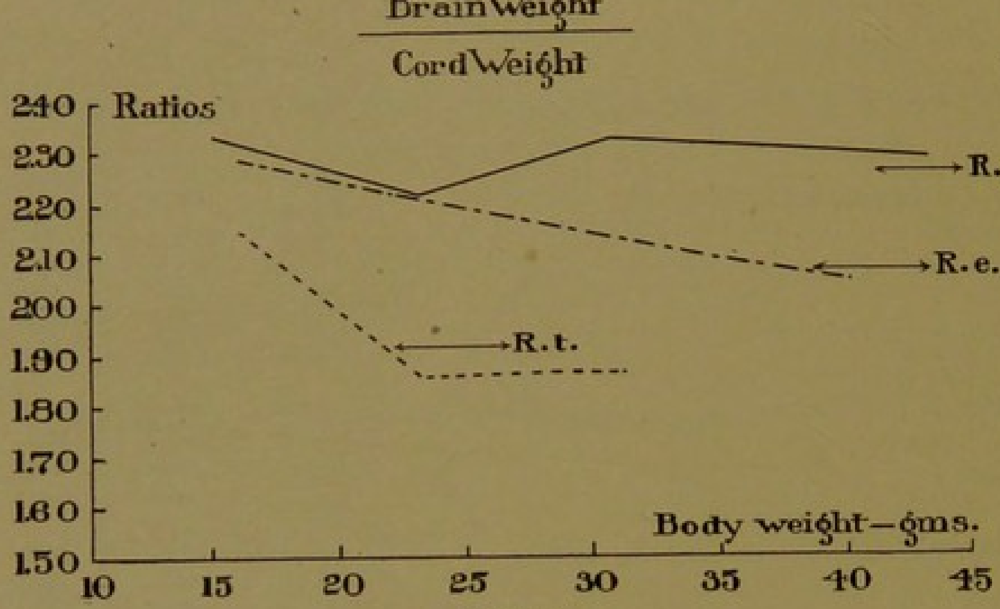

CHART 4.

Showing the ratios of brain weight to spinal cord weight.
R. p. = Rana pipiens.
R. e. = Rana esculenta.
R. t. = Rana temporaria.

TABLE 13.

Showing the percentage of water in the brain and in the spinal cord. Averages from groups of three.

	Body Weight.	Percentage of Water in	
		Brain.	Spinal Cord.
R. pipiens	14.9	84.5	80.2
	23.2	84.7	80.1
	30.8	85.0	80.5
	43.2	85.7	81.2
R. esculenta	15.9	83.6	78.6
	22.0	83.3	78.9
	35.0	83.3	78.4
	40.2	83.2	78.3
R. temporaria	15.9	82.7	78.6
	23.1	82.3	77.2
	28.0	82.1	76.9
	31.3	81.6	77.8

In addition to the several weights, a determination of the percentage of water was made in the case of both the brain and spinal cord. The method has been described already on p. 128.

The condensed results are given in Table 13.

On putting the data in Table 13 in the form of curves (Chart 5) it becomes evident at a glance that the percentages found in the three species are different, and also that they follow the order of the weight of the entire central nervous system and of the brain.

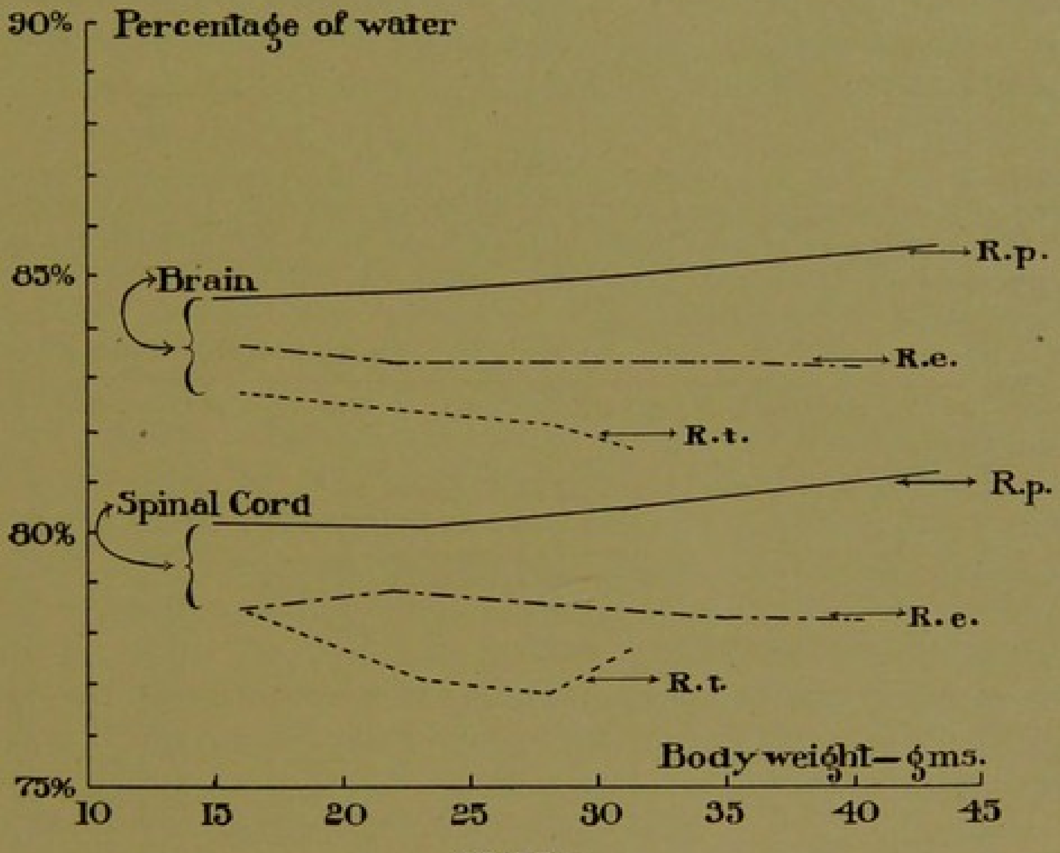

CHART 5.
Showing the percentage of water in the brain and in the spinal cord.
R. p. = Rana pipiens.
R. e. = Rana esculenta.
R. t. = Rana temporaria.

These differences in a character in which we might expect a high degree of similarity, call for some comment touching the trustworthiness of the results.

First, as to R. pipiens; the percentages in this species are the highest. The best evidence for their general correctness is furnished by the following table, extracted from the unpublished observations (1901) previously mentioned. At each date given in Table 14 the percentage of water was determined in a group of eight frogs (4 males and 4 females) and seventeen such records are here reported. The body weight of the frogs in each group was the same, that is, 25 grams, approximately.

The range in the entire series here given is for the brain from 84.2 per cent to 86.2 per cent, and for the spinal cord from 78.8 per cent to 81.6 per cent, while the average of the four entries for the month of August, is for the brain 85.1 per cent and for the spinal cord 80.5 per cent. These are very close to the values for the second and third groups of R. pipiens (with a body weight near 25 grams) as given in Table 13.

TABLE 14.

Percentages of water in the brain and in the spinal cord of R. pipiens. Average body weight is 25 grams. Each entry is based on a determination for eight specimens (4 males and 4 females). From an unpublished study on the influence of season made in 1901.

Date.	Percentage of Brain.	Water in Cord.
June 5	86.2	81.2
12	85.8	79.5
19	85.4	81.3
26	85.8	81.2
July 1	85.7	81.2
8	84.3	79.8
15	84.4	79.8
22	85.1	80.9
29	85.2	81.2
Aug. 5	84.6	79.6
12	85.2	80.6
19	85.1	81.6
26	85.3	80.0
Sept. 2	84.8	80.3
9	84.2	78.8
16	85.6	81.2
23	84.4	80.1

It seems probable from this comparison that we have obtained a generally correct value for the percentage of water in R. pipiens. In the case of R. temporaria, the specimens were dried at Liverpool, but not weighed until I reached Zurich. There they were further dried for 24 hours in the oven that was also used for drying the R. esculenta material, and then were weighed. They were found to give (see Table 13) the smallest percentages of water. This naturally raised the question as to whether they had been completely dried. The evidence that the drying was complete is only indirect. It is as follows:

The brains and spinal cords of R. esculenta dried and weighed at Zurich, were left in the original weighing bottles from the summer of 1904 to the spring of 1907, when, after careful redrying, they were weighed at the Wistar Institute in Philadelphia.

The last series of weighings made at Philadelphia, differed from those made in Zurich in 1904, by an average of plus 0.1 per cent. The fact that there was a trifling *gain*, is probably to be credited to the different balances used. But whatever the explanation of this gain may be, it seems to show that the drying in Zurich was complete, and thus to warrant the use of the values for R. esculenta and R. temporaria as entered in Table 13.

Assuming that in any given locality, the humidity of the atmosphere might be a factor influencing the amount of water in the body of a frog, I made an examination of the humidity records from July 1 to September 1, 1904, taken by the weather bureaus at Liverpool, Zurich and Chicago. For the data with which to do this, I am indebted to the officials of the U. S. Weather Bureau, whose courtesy I desire to acknowledge with thanks.

The matter is far too complex to permit us to make here more than the most general statements, but I feel justified in stating that the humidity conditions at Liverpool in July, 1904, and at Zurich and Chicago in August, 1904, were not unusual. Further, that broadly-speaking, the humidity is greatest at Liverpool, intermediate at Zurich, and least at Chicago. It is to be noted that the percentage of water in the several species follows the inverse order, being most in the Chicago specimens, where the humidity is lowest, and least at Liverpool, where it is greatest; a suggestive result which invites further inquiry.

Two more comments are however desirable before leaving this general topic.

From previous studies, we should expect that the percentage of water in the brain and in the spinal cord would diminish with increasing age, for the measure of which we here take the body weight.

This decrease is clear and regular for the brain of R. esculenta and R. temporaria, is indicated though less regular, in the case of the spinal cords of these two species, but in R. pipiens is regularly reversed in the case of the brain, and irregularly reversed in the case of the spinal cord. This makes it highly probable that some disturbing influence has modified the percentage of water in the brain and cord of R. pipiens, so as to mask the effect of age (size), but it is to be added that the disturbance thus produced is relatively small, and not sufficient to affect the distinctive differences between R. pipiens and the species here compared with it.

If the average values for the percentage of water in the brain and spinal cord of the three species are calculated from Table 13 we obtain the following:

TABLE 15.

Average values for the percentage of water in the brain and spinal cord of all three species, together with the difference between that for the brain and for the spinal cord in each species, and the relative amount of water in the spinal cord, that in the brain being taken as a standard.

	Percentage of Water in		Difference.	Percentage Value of Cord Determination.
	Brain.	Sp. Cord		
R. pipiens	84.97	80.50	4.47	94.7%
R. esculenta	83.35	78.55	4.80	94.2%
R. temporaria	82.17	77.62	4.55	94.4%

It appears from this table that the absolute differences in the percentage values for the brain and cord are similar in the three species, and that the determinations for the brain being taken as the standards the relative values of the determinations for the spinal cord are about alike, ranging from 94.2 per cent R. esculenta, to 94.7 per cent R. pipiens. The similarity in these relations speaks for the correctness of the general results.

In this connection it is natural to enquire how the weight relations of the central nervous system or its parts, might be affected if the percentages of water in R. esculenta and R. temporaria were raised to that found in R. pipiens. Calculations have been made, and the results show that the superiority of the entire central nervous system and of the brain in Rana pipiens would be diminished only slightly. On the other hand, the weights of all the spinal cords would be brought together, and R. temporaria given the heaviest cord.

Moreover, in general, the weight values in the two European species would be brought closer to one another.

These alterations would however not essentially modify any of the differences on which we have had occasion to lay emphasis.

For the foregoing comparison of the central nervous system and its parts, together with the determination of the percentage of water, data on all three species have been available. But before commenting on the results just given, I wish to present some observations based on the comparison of two species only.

These additional observations are on the peripheral nervous system and relate first, to the number of medullated fibers in the spinal nerve roots; comparing R. esculenta with R. pipiens (there being no corresponding observations on R. temporaria). Second, to the length of the internodal segments; comparing R. temporaria with R. pipiens (there being no corresponding observations on R. esculenta).

The number of medullated nerve fibers in the spinal nerve roots of R. pipiens compared with the number in R. esculenta.—In a female R. pipiens weighing 48.2 grams, HARDESTY ('99) reported 14,582[2] medullated nerve fibers in both roots of the ten spinal nerves of one side. This was a much larger number than had been found by BIRGE ('82) in a specimen of R. esculenta of greater body weight. To reduce BIRGE's figures for the specimen of R. esculenta, weighing 63 grams, to those for a specimen weighing only 48.2 grams, we have proceeded as follows:

The smallest frog in BIRGE's series, with a body weight of 1.5 grams, in which he enumerated 2992 motor fibers in the ventral spinal roots of one side, was selected as one limit, and to this frog the same proportion of sensory fibers as was found in the 63 gram specimen, was allotted, a concession which probably makes the number of sensory fibers somewhat too large.

The number of fibers corresponding to each gram of body weight between 1.5 grams and 63 grams was then determined. By this method, it was found that when the number of fibers in the spinal nerves of the 63 gram frog was reduced to the number for a 48.2 gram frog, it amounted to 92.8 per cent of that found in the 63 gram frog, or 8925 fibers. Thus the difference between the two species is (14,582 − 8925) 5657 fibers, or put in another way, R. esculenta has only about 61 per cent as many medullated nerve fibers in the spinal nerve roots as has R. pipiens. On reducing the original observations of BIRGE for the number of fibers in the

[2] By a clerical error the number was printed on p. 78 (HARDESTY '99) as 14,783. It should be 14,582, and consequently I shall use the corrected number subsequently, even when referring to HARDESTY's paper.

several nerve roots to 92.8 per cent of their value, we obtained the following table.

TABLE 16.

Number of medullated nerve fibers in the dorsal and ventral roots of the spinal nerves of one side.

Number of Nerve.	R. pipiens (Hardesty) 48.2 Grams.		R. esculenta (Birge) Original Weight 63 Grams Reduced to 48.2 Grams.	
	Dorsal.	Ventral.	Dorsal.	Ventral.
II	132	1045	115	727
III	2496	1478	1530	905
IV	329	379	245	446
V	371	163	179	98
VI	299	127	208	106
VII	350	251	171	148
VIII	1108	377	521	132
IX	2108	1295	1022	807
X	1171	721	921	409
XI	61	321	38	197
Totals	8425	6157	4950	3975
Sums	14582		8925	
Ratios	1.36—1		1.24—1	

When the data in Table 16 are thrown into the form of curves, we have the relations exhibited in Chart 6.

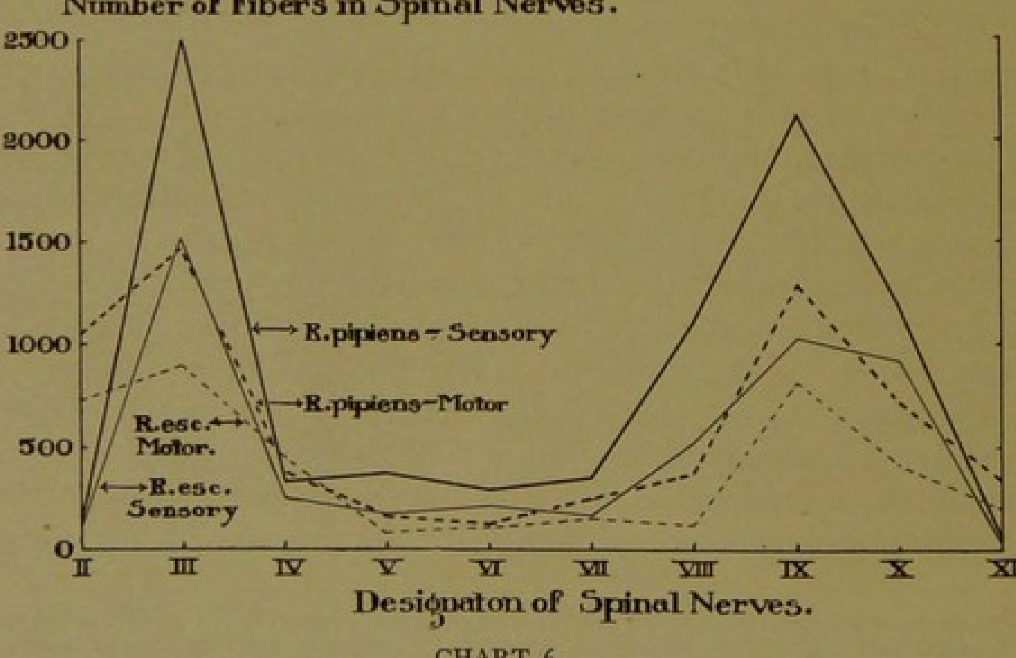

CHART 6.

Showing the numbers of medullated fibers in the spinal nerves of Rana pipiens and Rana esculenta.

From a study of Chart 6 we see that the form of the curves for the two species is very similar, even in details, although there are two evident differences.

In the first place, R. pipiens has regularly more fibers in each instance except in the ventral root of the IV spinal nerve, in which it has only 379 fibers against 446 in R. esculenta. In the second place, there is in R. pipiens a marked excess of fibers in the dorsal root of the IX nerve. In R. esculenta, the corresponding excess is distributed between the IX and X nerves. As a second specimen of R. esculenta, weighing 23 grams, examined by BIRGE ('82) shows a similar distribution, the possibility is suggested that this arrangement may be characteristic for R. esculenta.

The foregoing Table 16 also brings out the fact that the number of sensory, as compared with the number of motor fibers, is relatively greater in R. pipiens. Thus

	Motor.	Sensory.
R. pipiens	1	1.36
R. esculenta	1	1.24

However a further analysis of this relation shows that in the lumbar nerves VIII, IX and X the proportions of motor to sensory are nearly alike in the two species.

	Motor.	Sensory.
R. pipiens	1	1.833
R. esculenta	1	1.827

Moreover for the III nerve these proportions have been assumed as similar (see HARDESTY '99, p. 78), so that the difference which is found when the total number of fibers is compared, must depend on differences in this relation, which exists in the roots of the II, IV, V, VI, VII - - - XI nerves.

The ratio in this group of roots is

	Motor.	Sensory.
R. pipiens	1	0.674
R. esculenta	1	0.555

Thus showing R. pipiens as superior in this last group, although in both species the ratio is less than unity.

As a consequence of these relations, it appears that while R. pipiens has everywhere a better sensory innervation, because there are absolutely more afferent fibers present for the same area of skin and weight of muscle, the relative sensory supply is superior only in the head and trunk, but not in the skin and muscles of the limbs.

Such anatomical differences as these just described, suggest corresponding physiological differences between the two species. In pursuance of this suggestion, I sent out a letter of inquiry to my physiological colleagues in May of 1907. I take this opportunity of thanking my numerous correspondents for their courteous replies, but at the same time must report with regret that there do not appear to be any data bearing on the possible physiological differences, concerning which inquiry was made. The number of medullated nerve fibers in the spinal nerve roots of R. temporaria, has still to be determined.

A comparison of the length of the internodal segments in the fibers of the sciatic nerve of R. pipiens and R. esculenta.—As the heading indicates, the comparison will here be limited to fibers from one nerve. Boycott ('04) has determined the length of the internodes in fibers taken from the sciatic nerve just at the point where it divides into the nervus tibialis and the nervus peroneus. The length of the internodes at this locality depends on two factors, first the size (length) of the frog, and second the diameter of the fiber; the internodes becoming longer, the larger the frog and the greater the diameter of the fiber examined.

By a study of specimens of R. temporaria of different lengths, Boycott was able to show that the average length of the internodes of fibers of all diameters taken from the sciatic, increased in the same proportion as the length of the sciatic, the curves representing the two series of measurements running parallel. Accepting this result, it is possible to calculate the average length of the internodes at this point for frogs of different sizes.

Below is given a table containing the data on the six largest specimens of R. temporaria examined by Boycott ('04, p. 375). These are arranged in the order of increasing body length, the measurement being made from the tip of the nose to the end of the urostyle.

The body weight here given was taken as usual. The length of the sciatic in millimeters is defined by Boycott (*loc. cit.*, p. 371), as follows: "The upper end has been taken throughout as the point of emergence from the vertebræ of the upper of the two larger branches of the plexus.

There is no good fixed point for the lower end, the one which has been adopted as the cut end, obtained by cutting across the leg through the knee joint at right angles to the axis of the leg when it is in full extension."

TABLE 17.
R. temporaria.

No. of Specimen.	Body Weight in Grams.	Length of Sciatic in mm.
XXI	15.10	46.0
XXII	20.15	45.0
XXIII	16.45	45.5
XXIV	18.15	50.0
XXV	20.80	49.0
XXVI	24.80	53.5
Average	19.20	48.2

To compare with these, we have observations on four specimens of R. pipiens, made by Mr. TAKAHASHI[3] in the Neurological Laboratory of the University of Chicago.

The specimens examined by TAKAHASHI, and in which the internodes were studied in the same locality as that selected by BOYCOTT, were four in number, and the measurements made on them are given in the following table:

TABLE 18.

No. of Specimen.	Body Weight in Grams.	Sciatic Length in mm.* (Calculated.)
III	26	49.
V	34	53.3
VI	37	60.2
VIII	63	65.8
Average	40	57.3

* This measurement was not made by Mr. TAKAHASHI, but has been calculated from other data in his tables.

In accordance with BOYCOTT's results, we should expect in this series of R. pipiens, with an average sciatic length of 57.3 mm. to find longer internodes than in the series of R. temporaria, with an average sciatic length of only 48.2 mm., but on the contrary, the internodes in R. pipiens are much shorter. To make the comparison fair however it is necessary to reduce the measurements on R. pipiens to the measurements of the R. temporaria series,

[3] Mr. TAKAHASHI kindly allows me to use the data from his forthcoming paper on the internodes in R. pipiens.

which is taken as the standard. To do this, we divide the observed values for the R. pipiens series by 1.188, since 57.3 mm., the average length of the sciatic in the series of R. pipiens is 118.8 per cent of 48.2 mm., the average length of the sciatic in the series of R. temporaria.

The observations thus reduced to the same standard are given in the following table.

TABLE 19.

Giving the lengths of the internodal segments in μ on the medullated fibers of the sciatic nerve, for frogs with a sciatic length of 48.2 mm., arranged according to the diameter of the fibers.

Number of Observation	R. temporaria (Boycott) Length of Internodes	Diameter of Fibers.	R. pipiens (Takahashi) Length of Internodes.	Number of Observations	Relative Value of Lengths in R. pipiens.
In all about 1050	767	5–5.9μ	500	159	65%
	*1186	6–6.9μ	586	107	49%
	1102	7–7.9μ	705	92	64%
	1159	8–8.9μ	826	16	71%
	1288	9–9.9μ	917	4	71%
	1399	10–10.9μ	929	47	66%
	1416	11–11.9μ	942	14	66%
	1536	12–12.9μ	1027	5	66%

* As will be seen, Boycott's value for the length of the internodes in fibers 6–6.9μ in diameter, is plainly aberrant, and therefore the percentage value for the internodes of fibers having this diameter in R. pipiens, is excluded from the general average.

The foregoing table shows that when grouped according to diameters, the internodal lengths in R. pipiens range between 64 per cent and 71 per cent of that in R. temporaria, the average being 67 per cent.

It follows from this that R. pipiens has three sheathing cells on a fiber, where R. temporaria has only two, and therefore more cells in the length of the sciatic.

Consequently R. pipiens has the finer and more complete construction, although it is not possible to say what physiological advantage goes with this difference in structure. There are no observations on R. esculenta to compare with those just given.

Conclusions. From the observations presented, we conclude that the three species studied are similar in general form and proportions, but that R. pipiens has:

1. A heavier central nervous system.

2. A heavier brain and spinal cord.

3. A heavier brain in proportion to the weight of the spinal cord.

4. A greater percentage of water in both the brain and spinal cord.

5. A larger number of both sensory and motor medullated fibers in the spinal nerves (when compared with R. esculenta).

6. A slightly greater proportion of sensory fibers in the spinal nerves (when compared with R. esculenta).

7. Shorter internodes, and therefore a greater number of sheathing cells (when compared with R. temporaria).

With the possible exception of the percentage of water, the interpretation of which is not yet clear, all these characters may be counted to the credit of R. pipiens as indicating a higher development of its nervous system, and if we may make these characters a basis for physiological predictions, we should expect the American leopard frog, R. pipiens, when compared with the European, R. esculenta and R. temporaria, to give (1) more perfect general reactions associated with (2) less perfect reflex ones, and also to be both (3) stronger and (4) more sensitive.

APPENDIX.

The observations of Fubini, '81.

In 1881 FUBINI published, under the title "Gewicht des Centralen Nervensystems im Vergleich zu dem Körpergewicht der Thiere bei Rana esculenta und Rana temporaria," a study of the weight of the brain and spinal cord in the two European species commonly used for experiment. His data are comprised in eight tables, each sex being represented by four tables, and the records on twelve specimens entered in each table. His main object in this study was to show that in the female frog, the weight of the central nervous system was less than in the male. As I have elsewhere explained (DONALDSON and SCHOEMAKER, '00), he does not show this, having fallen into error by reason of his failure to appreciate that the relative weight of the central nervous system diminishes with the increasing body weight of the frog.

Despite this failure in the interpretation of his records, it was desirable to examine further his original tables in order to determine what he had recorded concerning the weight of the brain and spinal cord.

The weight of the brain and of the entire central nervous system is given in all the tables. The weight of the spinal cord can be obtained therefore by subtracting the former from the latter. Having the weight of the brain and spinal cord, we can find the ratio between them.

There are moreover two tables, one for each species, in which we have the body weights of males to compare with the weight of the central nervous system. In the other six tables, the body weights for the males (two tables) are given "after evisceration" and for the females (four tables) without correction for ova. In these cases the body weights can only be estimated.

These data have been carefully worked over, with a view to determining how they compare with my own.

In the first instance, FUBINI'S observations on the brain weights in unopened males of R. temporaria, are closely similar to mine. He obtains, however, weights for the spinal cord nearly double mine; thus his brain cord ratio is abnormally low. This is shown in the following table.

TABLE 20.

Showing the ratios of brain weight to the cord weight as determined by FUBINI, and by me.

Rana temporaria

DONALDSON REPETITION OF TABLE 12. BODY WEIGHT.	RATIO.	FUBINI BODY WEIGHT.	RATIO.
15.9	2.15	23.1 (male observed)	1.26
23.1	1.86	25.0 (male estimated)	1.70
28.0	1.87	31.0 (female estimated)	1.14
31.3	1.87	35.0 (female estimated)	1.77

Rana esculenta.

DONALDSON REPETITION OF TABLE 12. BODY WEIGHT.	RATIO.	FUBINI BODY WEIGHT.	RATIO.
15.9	2.29	20.0 (male estimated)	1.76
22.0	2.22	28.2 (male observed)	1.25
35.0	2.09	30.0 (female estimated)	1.80
40.2	2.05	35.0 (female estimated)	1.62

In the same way his observations on the weight of the brain in R. esculenta run only 10 to 15 per cent below mine, but the weights for the cords are much higher than mine, and the ratios as seen in the above table, are quite impossible and hopelessly irregular.

In view of these relations of brain to cord, I conclude that FUBINI's results are in general not trustworthy, and therefore do not require further discussion.

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