POTASSIUM, SODIUM AND WATER INTERCHANGE IN IRRITABLE TISSUES AND HAEMOLYMPH OF AN OMNIVOROUS INSECT, PERIPLANETA AMERICANA

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Muscle and nerve are commonly thought of as functioning optimally in an extracellular environment whose potassium concentration is low compared to that intracellularly. There is, however, evidence that in certain insects the tissue environment may contain very large amounts of potassium. Thus, it has been claimed that in insect haemolymph in general "sodium seems always to be replaced largely by potassium" (Wigglesworth, '39), and recently it has been shown that while the Na:K ratio in the haemolymph of carnivorous insects is greater than unity, it is less than unity in phytophagous insects (Boné, '44). For anyone interested in the physiology of muscle and nerve such findings can act as adequate stimuli for quantitative chemical studies beyond those presently available in the literature. In response, this report concerns itself with studies on an omnivorous insect; preliminary work on phytophagus insects is reported in the paper following this one (Tobias, '48).

Toward the end of understanding the situation in Periplaneta americana measurements have been made of water, sodium and potassium in haemolymph, serum, nerve cord and

muscle with the insects on a mixed diet (Purina dog chow, apple and water), on a high potassium diet (lettuce and water) and at fixed times after the ingestion (oral intubation) of measured volumes of known concentration KCl solutions.

METHODS

The roaches, Periplaneta americana, which have been used throughout, were collected at random from the basements of 2 University buildings. Precapture history and nutrition are therefore unknown, but all the animals were kept for at least 2 weeks on a diet of Purina dog chow checkers, apple and water before being used.

Oral administration of measured solutions is readily accomplished, after pinning the supine insect, through the wings, to cardboard, by gently inserting the blunted tip of a 27 gauge hypodermic needle through the mouth into the gut. Volumes up to at least 0.2 cm^3 per gram of body weight can then be injected from a 0.25 cm^3 tuberculin syringe with relatively rare regurgitations.

To obtain tissues for analysis the supine insect is fixed in position by pins passing through the pronotum and posterior abdomen. The antennae are amputated and the head and legs immobilized with pins. Next the ventral surface of the neck and thorax is cleaned with a bit of distilled water on a swab and dried. A small incision (0.5–1 mm long) is then made through the thin exoskeleton where the prothoracic leg joins the thorax. The haemolymph which wells up is drawn by capillarity into melting point tubes (0.5 mm i.d.). Bleeding can be speeded and increased by gentle massage of the abdomen, and, on the average, 5–20 mg of haemolymph are obtained from each insect. If whole haemolymph is to be analyzed it is expelled from the tubes into previously weighed, small, platinum combustion boats, reweighed, dried and analyzed as described below. If serum only is wanted, the capillary tube is sealed at one end, with care not to warm the contents, and centrifuged. The cells go to the bottom which can be broken off with a file, and the clear serum is then blown into a boat.

After haemolymph has been collected and the prothoracic and mesothoracic legs are amputated up to the coxae, the coxal muscle is laid bare and excised with a jeweler's forceps and iris scissors. With care 30 mg of muscle can be obtained.

Nerve cord is excised and weighed as described elsewhere (Tobias, Kolross and Savit, '46).

All tissues are put in weighed platinum boats and dried for 24 hours at 105°C. Water content is calculated from weight lost during drying. Tissue samples totalling up to 50 mg wet weight, pooled from 5–10 animals, serve well for the analyses described below.

For sodium, potassium and magnesium analyses the dried tissue is ashed by heating at 500°C. in a muffle furnace for 18–24 hours. The boat containing the ash is then put in a 25 cm³ glass stoppered Erlenmeyer and is flooded with 5 cm³ of 0.1 N-HCl. When the ash is dissolved, 10 cm³ of water are added. Such a flask fits directly onto the suction intake of the photometer atomizer, no further transfer of material being necessary.

Sodium and potassium have been analyzed for with the Perkin-Elmer, model 18 flame photometer. This early model of a currently improved instrument leaves much to be desired in terms of stability. The general principles have been described elsewhere (Barnes, Richardson, Berry and Hood, '45; Hald, '46; Berry, Chappell and Barnes, '47). During the present experiments there has been no extensive investigation of the instrument per se; it has, however, been used with rather rigorous controls and has given satisfactory results. Standard curves were made with known solutions of sodium and potassium chloride, and, following the early experiments, the unknown, after being located on the standard curve was checked several times against the nearest standard. When a given unknown was measured in terms of different standard curves it was possible, with care, to reproduce results within 2–7% having total quantities of the order of from 50 down to 5 µg of cation per cm³ of solution. It is important to emphasize that frequent comparisons with the standard are necessary

because of instability of the readings. A possible source of error stems from the action of interfering ions (Berry, Chappell and Barnes, '47). Except for sodium, potassium and magnesium the composition of the unknowns was not known precisely. Therefore allowances could not be made for possible interference by other ions. It was however determined that sodium and potassium, in the amounts present, did not interfere with each other; nor did magnesium or the amount of HCl used interfere with Na or K analysis. Since other ions were probably present in rather small quantities it seems doubtful that there was serious interference. As will be apparent from the magnitude of the standard error values, the method, as used, is not satisfactory for very small differences. Fortunately the changes encountered were relatively large. All data have been analyzed according to the T test (Fisher, '46), and their significance is stated in the text.

Magnesium was measured in an aliquot of solution remaining after sodium and potassium analysis, by the titan yellow method of Garner ('46).

The results of tissue analysis are expressed in terms of cation per unit wet and dry weight, and in molarity, calculated as if all of the ion in question were in solution in the tissue water. The latter quantity is stressed because of its implicitly greater importance for the questions at hand.

RESULTS

Observations on insects fed a complete diet

For the purpose of establishing normal values, insects were fed ad libitum on Purina dog chow checkers, apple and water for at least 2 weeks prior to tissue analysis. The guaranteed analysis of Purina checkers is:

Crude protein not less than 21.0%
Crude fat not less than 4.0%
Crude fiber not more than 6.0%
Nitrogen free extract not less than 46.0%

Ingredients: meat meal, dried skimmed milk, wheat germ, dried beet pulp, corn grits, cereal feed (from corn and wheat), dried raisins, soybean oil meal, molasses, riboflavin supplement, brewers' dried yeast, vitamin A and D feeding oils, steamed bone meal, 1% iodized salt.

TABLE 1

Water, sodium, potassium and magnesium content of several tissues under various experimental conditions, Periplaneta americana.

TISSUE AND EXPERIMENTAL CONDITION	WATER		Na			K			Mg		
	No. anls.[1]	%	No. anls.[1]	gm/kg wet	dry	No. anls.[1]	gm/kg wet	dry	No. anls.[1]	gm/kg wet	dry
Purina dog chow and water diet											
Whole haemolymph	9	89.1 ± 1.3	5	3.5	31.9 ± 8.8	5	1.0	8.7 ± 1.7	4	0.07	0.6
Haemolymph serum	8	93.6 ± 1.2	4	2.3	36.0 ± 8.3	4	0.6	9.9 ± 2.3	5	0.04	0.6
Nerve cord with sheath	13	75.5 ± 0.9	9	1.4	5.6 ± 0.6	9	4.2	17.3 ± 1.4	2	0.07	0.3
Muscle	8	74.1 ± 1.1	7	0.8	3.0 ± 0.3	6	3.3	12.6 ± 1.0	6	0.18	0.7
Leaf diet for 12–18 days											
Haemolymph serum	5	94.8 ± 0.3	5	2.6	50.0 ± 6.4	5	1.0	19.1 ± 1.9			
Nerve cord with sheath	5	79.7 ± 3.2	5	1.9	9.3 ± 2.5	5	5.6	27.4 ± 6.1			
Muscle	6	77.5 ± 0.9	6	0.7	3.2 ± 0.3	6	3.2	14.2 ± 1.2			
1.4 N-KCl orally 15–45 mins. before —											
0.04 cm³ per gram											
Haemolymph serum	7	92.7 ± 1.1	7	3.0	40.6 ± 6.3	7	1.4	19.3 ± 3.7			
0.12 cm³ per gram											
Haemolymph serum	10	92.7 ± 0.8	10	2.8	38.7 ± 4.6	10	1.8	24.6 ± 5.4			
Nerve cord with sheath	10	80.5 ± 2.3	10	1.4	7.1 ± 1.5	10	6.8	34.9 ± 4.9			
Muscle	10	76.7 ± 2.0	10	0.6	2.4 ± 0.3	10	3.9	16.8 ± 0.8			
Deprived of food and water 24 hours											
Nerve cord with sheath	17		17		6.0 ± 0.3	18		15.4 ± 1.3			
Deprived of food and water 24 hours and poisoned with DDT											
Nerve cord with sheath	10	75.3 ± 1.6	9.	1.9	7.6 ± 0.5	15	3.6	14.7 ± 1.9			

[1] Each analysis on tissue pooled from 5–10 insects.   N.B. Statistical significance is given as ± S.E.

In addition, analysis showed: water — 8.2%, Na — 4.9 gm per kilogram wet weight or 5.2 gm per kilogram dry weight, and K — 4.7 gm per kilogram wet weight or 5.1 gm per kilogram dry weight.

The results of tissue analyses are shown in tables 1, 2, and 3. Though both sodium and potassium concentrations, on a molar

TABLE 2

Sodium, potassium and magnesium concentration in millimoles per liter tissue water.

TISSUE AND EXPERIMENTAL CONDITION	Na	K	Mg
Periplaneta americana			
On purina dog chow, apple water diet			
Whole haemolymph	169 ± 46	27.1 ± 5	3.0
Serum	107 ± 25	17.3 ± 4	1.7
Nerve cord with sheath	83.9 ± 9	140 ± 11	4.0
Muscle	45.6 ± 5	112 ± 9	7.4
On leaf diet for 12–18 days			
Serum	119 ± 14	26.9 ± 3	
Nerve cord with sheath	103 ± 26	178 ± 40	
Muscle	41 ± 3	106 ± 9	
Given 1.4 N-KCl orally 15–45 mins. before —			
0.04 cm³ per gram			
Serum	139 ± 22	38.9 ± 7	
0.12 cm³ per gram			
Serum	133 ± 16	49.2 ± 10	
Nerve cord with sheath	76 ± 16	218 ± 30	
Muscle	32 ± 4	131 ± 6	
Deprived of food and water for 24 hrs.			
Nerve cord with sheath	89.9 ± 4 [1]	125 ± 1 [1]	
Deprived of food and water for 24 hrs. and poisoned with DDT			
Nerve cord with sheath	106 ± 7	121 ± 15	
Frog [2]			
Plasma	108	2.6	
Human [3]			
Serum	157.7	5.4	1.6
Brain	81.8	93.6	7.1
Muscle	39.2	117.7	12.0

[1] Calculated on basis of 73.5% water.

[2] Fenn et al. ('35).

[3] Shohl ('39).

N.B. Statistical significance is given as ± S.E.

basis in tissue water, appear to be greater in whole haemo-lymph than in serum the differences found are not statistically significant (P 0.4 and 0.2). Therefore it would not be useful to calculate quantities of these elements in the haemocytes.

Concerning serum; it is clear that the blood serum in this insect contains much more sodium than potassium. In comparison with man, the serum potassium is seen to be much greater in the insect. For this reason, and also because sodium is lower than in the human, the sodium-potassium ratio, 6.2

TABLE 3

Sodium potassium ratios in Periplaneta and several vertebrates.
(Calculated in terms of millimoles of ion per liter tissue-water.)

INSECT	Na serum / K serum	Na cord / Na serum	Na muscle / Na serum	K cord / K serum	K muscle / K serum
On purina checkers, apple and water	6.2	0.78	0.42	8.1	6.5
Leaf diet for 12–18 days	4.4	0.87	0.35	6.6	3.9
0.12 cm^3 1.4 N-KCl per gram 15–45 mins. before	2.7	0.57	0.24	4.5	2.7

VERTEBRATE	Na brain / Na serum	Na muscle / Na serum	K brain / K serum	K muscle / K serum
Frog [1]	41.6			
Dog [2]		0.44	0.20	27.3	27.2
Human [3]	29.2	0.52	0.25	17.3	21.8

[1] Calculated from Fenn et al. ('35).
[2] Calculated from Eichelberger ('44).
[3] Calculated from Shohl ('39).

on a molar basis, is smaller than in man where its value is 29.2; in the frog the ratio is still greater, 41.6 (Fenn et al., '35). The 3 animals are therefore quantitatively very different, but in all there is a preponderance of serum sodium over potassium.

It is also seen that the potassium concentration is distinctly greater and the sodium concentration smaller in both nerve cord and muscle than in serum. In the case of nerve cord the difference cannot be shown to be statistically significant from

any single set of data, but the fact that cord sodium has been found to be less than serum sodium under all conditions measured (table 3) makes it highly probable that the difference is meaningful. Stated slightly differently, in this insect, as in mammals and other higher forms, the ratios of tissue potassium to serum potassium are greater than one, while those for sodium are smaller than one. The tissue-body fluid gradients therefore are directionally the same as in comparable mammalian tissues but are quantitatively very different; the potassium gradients are very much steeper in man than in insect, whereas the oppositely poised sodium gradients are steeper in the insect.

Effect of a leaf diet

For comparison with the above complete diet, and in the hope that it might be possible to mimic the Na:K ratio smaller than unity found in vegetarian insects (Boné, '44) (table 4), insects were placed on a diet of lettuce and water for 12–18 days before tissue analysis. The composition of lettuce (Sherman, '27) is:

Protein	2.27%
Fat	0.95%
Fiber	2.57%
Water	86.28%
Na	0.27 gm/kg wet weight; 1.97 gm/kg dry weight
K	3.39 gm/kg wet weight; 24.6 gm/kg dry weight

Actual food intake was not measured, but it is clear from comparison with the analysis of Purina checkers that the leaf diet contains much less sodium both in terms of wet and dry weight; it contains slightly less potassium on a wet weight basis, but much more on a dry weight basis.

Such a diet caused, on a molar basis, a probable increase of 56% in serum potassium concentration (P 0.08) (tables 1 and 2). Serum potassium concentration however was never made to exceed that of sodium which continued to be about 4.4 times greater (P 0.00). Also, as on a complete diet, tissue to serum potassium ratios were greater than unity, 3.9 and 6.6 respectively for muscle and nerve cord (P 0.01 and 0.00),

whereas tissue to serum sodium ratios continued to be less than one, 0.35 and 0.87 respectively. The only other change noted was an increase of 4.5% (P 0.03) in muscle water. The meaning of this water increase is not immediately apparent.

Effect of oral administration of KCl solutions

Evidence that there are highly efficient salt and water regulatory mechanisms in insects have been noted in the past (Lester and Lloyd, '28; Wigglesworth, '31; Boné, '43). The present experiments are however quantitatively rather interesting. Data given by Wigglesworth ('39) show the average osmotic pressure of insect haemolymph to be approximately equivalent to 0.18 M-NaCl (compare with 0.15 for mammals); such a solution is isosomatic with 0.18 M-KCl. In the experiments at hand the insects have been forced to ingest 0.12 cm^3 of 1.4 M-KCl (82 times as concentrated as serum) per gram of body weight. Because of the open circulation, haemolymph volume measurements are difficult to evaluate, but if one extrapolates from the figure of 6% of the body weight given for "circulating haemolymph" in Periplaneta fuligensis (Yeager and Tauber, '32) then the volume ingested was about 2 times that of the circulating haemolymph. On a basis of total body water (Merrill, Savit, and Tobias, '46), the volume ingested was about 17.5% (corresponding to one large drink of 8.8 liters for a 70 kg man). To recapitulate, the insects were forced to ingest a solution of about 8 times the osmotic pressure of haemolymph, containing potassium 82 times as concentrated as that in haemolymph, and in volumes equivalent to 17.5% of the total body water or 200% of the circulating haemolymph. Such quantities caused no symptoms or deaths in control animals observed for at least 72 hours. Comparison with mammals is not possible since, not surprisingly, no data have been found which deal with the instantaneous ingestion of comparable amounts of solution.

The effect of this procedure (tables 1 and 2) on haemolymph serum, at intervals up to 45 minutes after ingestion, was to raise potassium concentration from 17.3 to 49.2 mM (P 0.01).

Neither serum water nor sodium changed significantly (P 0.5 and 0.35). As a result, the serum sodium-potassium ratio was depressed to 2.7 as compared with values of 6.2 and 4.4 in the control and leaf fed insects.

Whereas the 2-week leaf diet failed to change tissue potassium or sodium significantly, the ingestion of large amounts of KCl did have effects. Nerve cord potassium was increased 1.6 times (P 0.03), but muscle potassium was only increased by about 26%, and this change was a doubtful one (P 0.1). In the case of sodium, however, there was no significant change in the nerve cord, whereas muscle sodium decreased by about 29% (P 0.04), a change approximately equivalent to that in muscle potassium. Nerve cord water increased by 6.6% (P 0.05), but there was no significant change in muscle water.

In terms of concentration gradients, it is seen (table 3) that the cord-serum potassium ratio was depressed about 44% and the muscle-serum ratio about 58% below normal. In the case of sodium, the muscle-serum ratio was depressed 43% and the cord-serum ratio about 28%, but this latter change could not be shown to be statistically significant.

Effect of poisoning with DDT

In the past, studies on the mechanism of action of DDT in insects have given no evidence of actual tissue destruction as measured in terms of total, body, non-protein nitrogen content (Merrill, Savit and Tobias, '46). Therefore it was felt that more subtle degrees of cell damage might be reflected in permeability changes with resultant shifts in tissue cation concentrations. Because neural tissue seems to be affected primarily (Tobias and Kollros, '46) the potassium and sodium content of nerve cord was measured after gross contact poisoning with DDT. Since DDT poisoned animals soon neither can feed nor drink, they and the controls were deprived of food and water from the time of poisoning until the time of dissection 24 hours later.

The results are seen in tables 1 and 2. DDT poisoning had no effect on nerve cord potassium or water. Cord sodium, however, may have increased somewhat (from 83.9 mM to 106 mM) (P 0.06). The biological meaning of this change is not clearly apparent, but it is interesting to note that the post-poisoning cord sodium concentration is just about in equilibrium with the normal serum sodium level. Serum sodium has not yet been measured after DDT poisoning, but this change suggests the possibility of some loss of semi-permeability for sodium.

DISCUSSION

To begin with, it is seen that in this omnivorous insect serum sodium is normally greater than serum potassium. This is contrary to what might be expected from the usual textbook statements, but is in keeping with the data of Boné who found serum potassium to exceed serum sodium only in the phytophagous insects (table 4). Compared to 2 very different species, it is seen that serum sodium is about the same as that in the frog and somewhat lower (about 32%) than that in man. The striking difference, however, is in serum potassium which is very much higher than that of either the frog or human, 6.6 and 3.2 times higher respectively. Primarily because of the very high potassium level, but also because of the lower sodium, the serum Na:K ratio in this insect (6.2) is much smaller than that in the frog (41.6) or in man (29.2). The matter of irritable tissue functioning in such a high potassium environment is discussed below.

Because Boné ('44) had shown a correlation between haemolymph Na:K ratio and dietary habit (ratio < 1 in phytophagous and > 1 in carnivorous insects) (table 4) it was hoped that the ratio in Periplaneta americana might be significantly modifiable by diet. However, a leaf diet maintained for 18 days, though it apparently elevated the serum potassium, came nowhere near depressing the ratio below unity. This suggests that a ratio smaller than unity, in vegetarian insects, is a func-

TABLE 4

Sodium and potassium content of insect haemolymph (from Boné, '44).

ORDER	GENUS AND SPECIES	STATE		DIET	Na eq./L	K eq./L	$\frac{Na}{K}$
Coleoptera	Cicindela maritima	Adult	C	Insects	0.162	0.009	18.0
	Dytiscus marginalis	Adult	C	Insects	0.133	0.010	13.3
	Tenebrio molitor	Larva	M	Meal, meat	0.086	0.045	1.9
	Agelastica alni	Adult	V	Leaves	0.017	0.047	0.36
	Melolontha vulgaris	Adult	V	Leaves	0.006	0.049	0.12
Hemiptera	Triatoma megista	Adult	C	Blood	0.133	0.005	26.6
	Cimex lectularius	Adult	C	Blood	0.139	0.009	15.5
	Garris najas	Adult	C	Insects	0.142	0.008	17.8
	Palomena prasina	Adult	V	Sap	0.022	0.042	0.52
Diptera	Stomoxys calcitrans	Adult	C	Blood	0.128	0.011	11.7
	Lucilia sericata	Larva	C	Meat	0.140	0.026	5.4
	Calliphora erythrocephala	Larva	C	Meat	0.148	0.037	4.3
	Pegomyia sp.	Larva	V	Leaves	0.026	0.058	0.45
	Chironomus sp.	Larva	A	Saprophytic	0.092	0.008	11.5
Hymenoptera	Apis mellifica	Larva	V	Pollen, honey	0.010	0.045	0.22
	Vespula germanica	Larva	M	Insects, fruit	0.048	0.041	1.2
	Tenthredinidae	Larva	V	Leaves	0.006	0.055	0.11
Lepidoptera	Bombyx mori	Larva	V	Leaves	0.014	0.035	0.40
	Pieris rapae	Larva	V	Leaves	0.011	0.038	0.28
	Vanessa urticae	Larva	V	Leaves	0.022	0.043	0.51
	Ephestia kuhniella	Larva	V	Meal	0.017	0.060	0.28
Orthoptera	Gryllotalph vulgaris	Adult	M	Insects, roots	0.174	0.011	15.8
	Locusta viridissima	Adult	M	Insects, leaves	0.083	0.051	1.6
	Stenobothrus stigmaticus	Adult	V	Leaves	0.061	0.062	0.98
	Carausius morosus	Adult	V	Leaves	0.021	0.025	0.84
Trichoptera	Chaetopteryx villosa	Larva	AV	Leaves	0.063	0.009	7.0
Archyptera	Calopteryx sp.	Larva	AC	Insects	0.158	0.009	17.5

C — carnivore; V — vegetarian; M — mixed diet; A — oxygen breathing aquatic forms.

tion of some metabolic peculiarity more fundamental than is mimicked by a simple change in diets.[2]

The acute ingestion of large quantities of KCl produced more striking increases in serum potassium with a further depression of the Na:K ratio, but doses compatible with life did not cause the ratio to fall below one. This is not to say that differently designed experiments could not accomplish this change in some fashion compatible with life. It would appear, however, that in this insect the normal situation is for serum sodium to exceed serum potassium even under conditions of severe overloading with potassium, implying a rather efficient ion regulating mechanism. In this same connotation it is interesting, from the comparative point of view, that while an oral dose of about 143 mg of KCl per kilogram in man raises serum potassium some 44% (Norn, '29), the dose given to the insect, though it was 87 times greater only raised serum potassium 6.3 times more.

If one now examines the concentration gradients of sodium and potassium existing between tissue and serum, several interesting facts are noted. First of all, it is seen that both nerve cord and muscle contain more potassium and less sodium than does serum. This is in line with experience in other forms. There is however a striking quantitative difference, the potassium gradient being much steeper in the mammal than in the insect and the sodium gradient being somewhat steeper in the insect. The biological meaning of this difference is not apparent. Methods of differentiating intracellular from extracellular substances are poor enough in mammals; certainly, in insects, with an open circulation and a quantitatively very different pattern of electrolyte distribution, methods cannot be freely extrapolated, and there is, at present, no

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[2] After this work had been done it was learned by communication from Dr. Boné that he had found no effect of diet on coelomic fluid Na:K ratio in Tenebrio molitor. He concluded (Boné, 47) that " . . . the association observed between the Na:K ratio in coelomic fluid and the diet is not the result of the chemical composition of the foodstuff acting during the actual life of a given insect. The ionic composition of the internal medium, as found now, is a species characteristic, maintained by mechanisms of mineral regulation."

basis for differentiating between intra- and extracellular substances. However, for the purposes of this discussion, the existence of an open circulation makes it seem likely that circulating haemolymph should resemble intercellular fluid if anything even more closely than does plasma in the mammal.

Therefore because of recent findings on the relation of tissue potassium to injury potential in frog muscle (Tobias, '48a), measurements of membrane potentials in insects are also being made. If the Bernstein ('02) theory of membrane potentials is correct, one might expect rather low potentials in the muscle and nerve cord of this insect.

Now then, concerning the changes in tissue cations as a result of changes in the serum: When serum potassium is significantly elevated there is an absolute increase in cord potassium which is greater than that in muscle. Because of the initially higher cord potassium, however, the tissue-serum gradients for both tissues decrease by more nearly the same amount on a per cent basis. Nerve, cord, therefore, seems rather freely permeable to potassium, the rate of uptake being in striking contrast to the much slower penetration reported for rat brain (Noonan et al., '41). It is, of course, possible that the rate of uptake in rat brain would be comparably rapid if tissue fluid K in the mammal could be comparably elevated. Insect muscle would appear to be considerably less permeable to this ion than is nerve cord. The reserve seems to be true of sodium, with muscle more freely permeable to its egress than is cord.

Little can be said about hydration effects. Although very large volumes of hypertonic solution were ingested, and rather free ion exchange occurred, there was remarkably little change in either muscle or nerve cord water concentration when referred to dry weight. This ability of insects to eliminate large volumes of water, in spite of ion interchange, has also been shown by Boné ('45) and others.

Finally, it is important to examine the data in the light of known effects of changing environmental potassium on tissue irritability in other forms. To begin with, irritable tissues in

the roach normally function in a medium whose potassium concentration, 17.3 mM, is just about at the level which is fatal, 15–20 mM (Winkler et al., '38), in mammalian serum. It is also clear that in this insect, since no untoward motor symptoms were noted, an in vivo serum potassium increase of as much as 284% (from 17.3 to 49.2 mM), a level far beyond that compatible with life in mammals, does not seriously alter neural or muscular function. One might explain this high tolerance, as it has been explained before (Heilbrunn, '43), on the basis that potassium may be bound in an innocuous form to some serum constituent such as amino acids. It may not be necessary, however, to so belabor the reasoning, at least in the case of nerve cord. Thus, using a nerve cord and sixth abdominal ganglion, synapse preparation from Periplaneta americana (Roeder, Kennedy and Samson, '47), it has recently been shown (Roeder, '47) that Ringer containing as much as 29 mM-K has no effect on conduction in vitro. Only minor threshold effects and inconstant block were caused by bathing the nerve cord in Ringers' solution containing potassium as concentrated as 38.4 mM; at about 48 mM conduction was reversibly blocked. These figures are in remarkably good agreement with the normally high levels of in vivo serum potassium and the still higher levels (49.2 mM) tolerated after KCl ingestion. There is, therefore, no need to argue that the high in vivo potassium is inactivated as bound potassium, since similar concentrations (29 mM) are tolerated in simple salt solution. One cannot be sure, of course, that a high tissue amino acid content is not responsible for the high tolerance even in vitro. In addition, it is interesting that the in vivo change in potassium tolerated by insect nerve cord is within the limits tolerated by frog sciatic nerve (Hertz, '47) and by squid giant axon (Curtis and Cole, '42) as judged by ability to propagate the action potential.

In the case of the excised heart of Periplaneta americana, the potassium concentration for optimum in vitro performance agrees with the serum analyses much better than in

the case of sodium. Thus, Yeager ('39) found the optimum saline for the heart to be 21.1 mM in potassium and 182 mM in sodium.

CONCLUSIONS

1. In the serum of Periplaneta americana sodium exceeds potassium. Because of the very high potassium level, and somewhat lower sodium, as compared to other animals, the Na:K ratio is small (6.2) compared to that in man (29.2) or the frog (41.6).

2. In this animal, irritable tissues, muscle and nerve, normally function in the presence of a high serum potassium level (17.3 mM) and can apparently function unimpaired in a serum whose potassium concentration is elevated to as high as 49.2 mM.

3. The experiments suggest that a serum Na:K ratio of less than unity, as is found in phytophagous insects, is a function of some metabolic or regulatory peculiarity more fundamental than is mimicked by a vegetarian diet.

4. In the omnivorous Periplaneta americana it was not possible to depress the serum Na:K ratio below unity with large oral doses of KCl. Very large doses of orally administered KCl are tolerated.

5. The concentration gradients of sodium and potassium existing between tissue and serum have the same directional orientation as in mammals; the potassium gradient is, however, much steeper in the mammal, the sodium gradient somewhat steeper in the insect.

6. Nerve cord accumulates excess potassium more readily than does muscle.

7. The excellent agreement between the high potassium level normally found to be tolerated in vivo and that found to be tolerated by excised nerve cord in Ringer solution obviates the necessity for explaining such tolerance on the basis that potassium in vivo exists largely as "bound" potassium.

8. The data suggest that poisoning with DDT may increase nerve cord permeability to sodium.

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