the R_2 doublet occupied the middle well. The separated doublets were examined under the dissecting microscope in the following 24 h for meiotic activation. If only 1 doublet became activated, it was considered the R_1 cell of the chain. When 2 doublets resulted in being activated the second one was always the R_2 cell of the chain. In experiment type b (fig.), after the removal of the albino cell, the chains were kept for 2 more hours in SMB without cycloheximide and then cut apart into their single components. The same procedure of isolation and inspection described above was followed. These chains served as a control. In experiment type c (fig.) the chains were treated as in experiment a, and left united in SMB for additional 2 h over the above incubation period in cycloheximide. Finally they were surgically separated, singly isolated as in experiment a, and inspected for meiotic induction. In all 3 types of experiments, chains with no activated doublet were discarded and not included in the results of the table. Thus, as the 1st doublets (R_1) were always activated attention was focused on the R_2 and R_3 doublets.

Results. As reported in the table, out of 30 chains considered, 9 R_2s were activated in experiment type a vs 24 R_2s and 20 R_2s in b and c respectively. Using the χ^2-test, a highly significant difference ($p < 0.01$) was found for R_2 cells between the a vs b and a vs c experiments. No significant difference was observed for R_2 and R_3 cells in b vs c.

Conclusions. 1. In a chain system of 3 doublets, when the R_1 cell is induced to undergo meiosis after removing the complementary singlet, induction always propagates into the other 2 doublets provided they remain united. 2. In similar chain systems the propagation of meiotic induction is reduced by cutting apart the doublets 2 h after the interruption of heterotypic union (experiment b). The number of activated doublets is lower than that observed by Miyake et al.[3] in analogous experiments. The discrepancy is most likely due to the elimination of the heterotypic effect from the chain after 2 h of union, which did not occur in Miyake's experiment. 3. The 2-h incubation period in cycloheximide soon after the cutting apart of the complementary cell (experiment a) significantly reduced the number of R_2 and R_3 cells induced to undergo meiosis when compared to the cells of the chains in experiment b. Protein synthesis, blocked by the inhibitor, does appear to be an indispensable process for the propagation of meiotic activation factor along the chain. This assumption is confirmed by the lack of a significant difference between the chains of experiments b and c, in experiment c, the resumed protein synthesis, after washing, permits an almost regular propagation (throughout the chain) although with a slight delay in the transference of meiotic activation factor. The reversible effect of the protein inhibitor cycloheximide, was similar to that observed in pairs of *Blepharisma* by Miyake et al.[4].

To conclude, these results strongly indicate the need for protein synthesis for the transfer of meiotic induction to other cells of chains in which one cell is already activated. As was previously found[4], the heterotypic cell union induces and maintains the synthesis of a protein required for meiotic activation. Our results do not exclude the possibility that the protein synthesized within the homotypic chain might be the same as that produced at the heterotypic cell union.

1 This work was supported by CNR, Programma finalizzato 'Biologia della Riproduzione'.
2 A. Miyake and J. Beyer, Exp. Cell Res. *76*, 15 (1973).
3 A. Miyake, M. Maffei and R. Nobili, Exp. Cell Res. *108*, 245 (1977).
4 A. Miyake, M. Tulli and R. Nobili, Exp. Cell Res. *120*, 87 (1979).
5 G. Santangelo and R. Nobili, J. exp. Zool. *218*, 121 (1981).
6 A. Miyake and H. Honda, Exp. Cell Res. *100*, 31 (1976).

Neutron activation analysis of saliva from the tick *Amblyomma variegatum*[1]

T.L. Devine

Institut für Angewandte Zoologie, Freie Universität Berlin, Haderslebener Strasse 9, D-1000 Berlin 41, West, and Kentucky State University, Frankfort (Kentucky 40601, USA), 9 December 1980

Summary. The hygroscopic saliva produced by fasting *Amblyomma variegatum* adults during prolonged exposure to dry air was found to contain sodium, potassium and chlorine in molar proportions of 1.1–1.7–3.8 respectively, or proportions by dry weight of 8, 21 and 41%. These values are quite different from those reported previously for saliva from recently fed ixodid ticks. The *A. variegatum* saliva contained, by dry weight, less than 5% sulphur and less than 1.5% each of phosphorus, calcium, magnesium and iron.

Certain terrestrial arthropods have the remarkable ability to obtain much of their water requirement by absorbing water from air having a relative humidity well below saturation[2]. Evidently water is collected from vapor by a hygroscopic secretion in some mites. In ixodid ticks this secretion is produced by the salivary glands[3,4]; in certain mites among the Acaridida a secretion of the supracoxal glands appears to be involved[5].

Analyses of these fluids are important in regard to their hygroscopic properties, but analysis is impeded by the minute amounts of secretion available. The elements sodium, potassium, chlorine and sulphur were identified in the hygroscopic salivary secretion of ticks[3,6]. The solids precipitated in the supracoxal gland ducts of *Dermatophagoides farinae* and *Tyrophagus putrescentiae* were found to contain potassium and chlorine[5,7].

In ixodid ticks only the copiously secreted saliva of recently-fed individuals has been analyzed quantitatively; it was found to contain sodium, potassium and chlorine at approximately 180, 10 and 140 µM/l respectively[8]. The salivary secretion of recently-fed ticks (which excrete water, ions and certain other components of their blood meal) might be expected to differ in composition from the hygroscopic salivary secretion associated with vapor uptake by nonfeeding ticks.

Materials and methods. During exposure to dry air after several weeks of fasting, *Amblyomma variegatum* adults produced a saliva from which the solids accumulated on the mouthparts. This solid residue was collected on an aluminium pan, weighed, and then kept in polyethylene vials. A 770 µg sample of the saliva solids was examined by X-ray fluorescence spectroscopy and then, after a brief

The principal reactions with reactor neutrons of the elements conceivably present in saliva and the quantities observed in salts and saliva after their irradiation

Material		Specific radioactivity		Quantities observed in 327 µg of saliva solids			
		Expected after 5 min irradiation (dpm/µM)	Observed in salt after 5 min irradiation (dpm/µM)	Radioactivity after 100 min irradiation (dpm)	Mass (µg)	Mass fraction	Moles (µM)
Detected							
Sodium	24 Na	1.5- 2.4 × 10^4		4.3 ± 0.1 × 10^5	26 ± 7	0.08	1.1 ± 0.3
Potassium	42 K	3.3- 6.5 × 10^3	6.7 ± 0.3 × 10^3	2.2 ± 0.05 × 10^5	68 ± 1	0.21	1.73 ± 0.02
Chlorine	38 Cl	6.7-10.0 × 10^4	6.8 ± 0.1 × 10^4	2.4 ± 0.2 × 10^6	135 ± 11	0.41	3.8 ± 0.3
Upper limits							
Phosphorus	32 P			1.06 ± 0.02 × 10^3			
from Cl	32 P	< 8	7.86 ± 0.05	6.0 ± 0.5 × 10^2 expected			
from P	32 P	2.2- 3.2 × 10^2		< 4.6 × 10^2	< 3	< 0.009	< 0.1
Sulphur	32 P	< 80	44.5 ± 0.04	< 4.6 × 10^2	< 15	< 0.046	< 0.5
	35 S			8.60 ± 0.01 × 10^3			
from Cl	35 S	7.4-14.0 × 10^1	92.4 ± 0.2	7.0 ± 0.5 × 10^3 expected			
from S	35 S	< 2	0.56 ± 0.04	< 1.6 × 10^3			
Magnesium	27 Mg	9.3-15.0 × 10^3	1.5 ± 0.7 × 10^4	< 8 × 10^3	< 4	< 0.012	< 0.15
Calcium	45 Ca	2.2- 3.2	3.3 ± 0.03	< 6	< 4	< 0.012	< 0.1
	49 Ca	5.0- 9.0 × 10^3	5.3 ± 1.1 × 10^3	< 7.5 × 10^3	< 17	< 0.052	< 0.4
Iron	55 Fe	2.3- 3.5		< 5	< 5	< 0.015	< 0.1
	59 Fe	1.4- 3.4		< 6	< 5	< 0.015	< 0.1

neutron activation, by gamma-ray spectroscopy (both by staff of the Hahn-Meitner Institut, Berlin), followed by beta-ray spectroscopy. A 2nd sample of the saliva solids, 327 µg, was analyzed in much greater detail by neutron activation and gamma-ray spectroscopy (at the Ohio State University Nuclear Reactor Laboratory) followed by beta-ray analysis (again at the Institut für Angewandte Zoologie). This 2nd sample was irradiated for 10 min, and its gamma-spectrum was determined with a resolution of 3 keV (FWHM). It was then irradiated for a further 90 min, and its gamma-spectrum was determined after decay times of 4 min, 38 min and 20 h. Beginning 15 days after irradiation, the beta radioactivity of the sample and of similarly-activated salt samples were monitored for 150 days in a liquid scintillation counter. The beta emitting radioisotopes were recognized by the half-times of their decay, and their radioactivities were evaluated by regression calculations on the count rates and decay times.

For each of the elements conceivably abundant in tick saliva, a lower limit for the specific radioactivity of each reaction product at the end of irradiation was calculated from values of the thermal neutron reaction cross-section[9] and the thermal neutron flux to which the material was exposed, 2×10^{11} cm^{-2} sec^{-1}. An upper limit of specific radioactivity was established by the additional contribution of the higher energy neutrons, 2×10^{11} cm^{-1} sec^{-1}, and their reaction cross-section[9]. Data for the more significant reactions are given in the accompanying table along with data on the saliva sample and on salt samples that were irradiated for 5 min in the same neutron spectrum as the saliva sample: 42.7 µM $Ca(NO_3)_2$, 20.1 µM K_2SO_4, and 9.35 µM $MgCl_2 \cdot 6\ H_2O$. Neither the X-ray fluorescence spectroscopy nor the neutron activation technique afforded an analysis of elements having a low atomic number, of which hydrogen, nitrogen, carbon and oxygen are of interest as probable components of the saliva solids.

Results and discussion. In the 770 µg sample of saliva solids, sodium, potassium chlorine and a small proportion of sulphur were identified by X-ray fluorescence spectroscopy. From its gamma-ray spectrum, 64% of the mass was accounted for; 380 µg (11 µM) of chlorine and 120 µg (4 µM) of sodium and potassium together. An upper limit of 40 µg (1 µM) of calcium was established by the absence of its characteristic gamma rays, and upper limits of 32 µg (1 µM) of sulphur and 0.3 µg (10 nM) of phosphorus were established from their beta radiation. In the 327 µg sample of saliva solids the more detailed gamma-ray analysis accounted for 70% of the dry mass: 68 ± 1 µg of potassium, 26 ± 7 µg of sodium and 135 ± 11 µg of chlorine. The upper limits for the other elements that were considered were 4 µg of magnesium, 5 µg of iron, 0.5 µg of cobalt, 4 µg of calcium, and 60 ng of iodine. The beta radioactivity identified in the sample was 8596 ± 12 dpm of ^{35}S and 1056 ± 19 dpm of ^{32}P. Of this ^{35}S, 90% would have been produced from the 135 µg of chlorine in the sample (and all of it would have been produced by 160 µg of chlorine). Of the ^{32}P in the sample, the 135 µg of chlorine would have produced 44% (but 160 µg would account for only 69%) and the rest must have been produced from 15 µg of sulphur, or 3 µg of phosphorus, or smaller amounts of each in the sample.

The molar proportions of sodium, potassium and chlorine in this saliva, produced by fasting *Amblyomma variegatum* adults during prolonged exposure to dry air (1.1:1.7:3.8) are quite different from those in saliva collected from 3 other species, either after feeding or by chemical stimulation (1.8:0.1:1.4), as reviewed by Hsu + Sauer[8].

1 Acknowledgments. I wish to thank Prof. W. Knülle for extending to me support granted by the Deutsche Forschungsgemeinschaft, and Dr D. Rudolph for providing the saliva solids.

2 E.B. Edney, Zoophysiology and Ecology, vol. 9. Springer, Berlin 1977.
3 D. Rudolph and W. Knülle, Nature, Lond. *249*, 84 (1974).
4 D. Rudolph and W. Knülle, in: Comparative physiology-water, ions and fluid mechanics, p. 97. Eds K. Schmidt-Nielson, L. Bolis and S.H.P. Maddrell. Cambridge University Press, Cambridge 1978.
5 G.W. Wharton and R.T. Furumizo, Acarologia *19*, 112 (1977).
6 D. Rudolph and W. Knülle, in: Recent advances in acarology, vol. 1, p. 375. Ed. J.G. Rodriguez. Academic Press, New York 1979.
7 G.W. Wharton, K.M. Duke and H.M. Epstein, Recent advances in acarology, vol. 1, p. 325. Ed. J.G. Rodriguez. Academic Press, New York 1979.
8 M. Hsu and J.R. Sauer, Comp. Biochem. Physiol. *52A*, 269 (1975).
9 G. Erdmann, Kernchemie in Einzeldarstellungen, vol. 6. Verlag Chemie, Weinheim 1976.