the conditions used for the data in Table 2 since it seems likely that the ratio of formation of these two products would be influenced by the physiological state. The data do, however, indicate that ammonia is a better substrate than glutamate for citrulline synthesis, whereas the reverse is true for glutamine synthesis.

The evolutionary precedent for a mitochondrial localization of glutamine synthetase in vertebrate liver was set in the elasmobranchs (*14*), where the enzyme functions in conjunction with the glutamine-utilizing carbamyl phosphate synthetase-III in the synthesis of urea for osmotic purposes. In amphibians, hepatic mitochondrial ammonia detoxication is via carbamyl phosphate synthetase-I. Immunochemical and other properties of carbamyl phosphate synthetases-I and -III suggest that the two are evolutionarily related (*16*). The utilization of glutamine synthetase for the intramitochondrial detoxication of ammonia appears not to occur in amphibians, even in those species that excrete a large percentage of their excretory nitrogen as uric acid (*17*). However, our data indicate that both systems may have been present in the stem reptiles that subsequently gave rise to the ruling reptile, avian, and mammalian lines of descent. Both carbamyl phosphate synthetase-I and cytosolic glutamine synthetase in mammalian liver show a heterogeneous distribution within this organ (*18*), so whether the same or different populations of tortoise hepatocytes contain both detoxification systems remains a question.

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### References and Notes

1. J. E. Vorhaben and J. W. Campbell, *J. Biol. Chem.* **247**, 2763 (1972).
2. J. W. Campbell and J. E. Vorhaben, *ibid.* **251**, 781 (1976).
3. C. Wu, *Biochim. Biophys. Acta* **77**, 482 (1963).
4. D. D. Smith, Jr., and J. W. Campbell, *J. Biol. Chem.* **258**, 12265 (1983).
5. A. S. Romer, *The Vertebrate Body* (Saunders, Philadelphia, 1956), p. 59; J. Z. Young, *The Life of the Vertebrates* (Oxford Univ. Press, London, ed. 2, 1962), pp. 386–399.
6. W. H. Dantzler, *BioScience* **32**, 108 (1982); G. C. Packard and M. J. Packard, *Science* **221**, 1049 (1983).
7. J. Mora, J. Martuscelli, J. Ortez-Pineda, G. Soberon, *Biochem. J.* **96**, 28 (1965); W. B. Baze and F. R. Horne, *Comp. Biochem. Physiol.* **34**, 91 (1970).
8. V. Moyle, *Biochem. J.* **44**, 581 (1949); F. Khalil and G. Haggag, *J. Exp. Zool.* **130**, 423 (1955); W. H. Dantzler and B. Schmidt-Nielsen, *Am. J. Physiol.* **210**, 198 (1966).
9. J. E. Vorhaben and J. W. Campbell, *Comp. Biochem. Physiol.* **62B**, 85 (1979).
10. ———, *J. Cell Biol.* **73**, 300 (1977).
11. D. D. Smith, Jr., J. E. Vorhaben, J. W. Campbell, *J. Exp. Zool.* **226**, 29 (1983).
12. J. G. Gamble and A. L. Lehninger, *J. Biol. Chem.* **248**, 610 (1973); S. Clarke, *ibid.* **251**, 950 (1976).
13. J. E. Vorhaben, D. D. Smith, J. W. Campbell, *Int. J. Biochem.* **14**, 747 (1982).
14. Tortoises are predominantly uricotelic in their nitrogen excretion (*8*). Hence their synthesis of urea could be to prevent water loss by raising the osmolarity of body fluids, such as occurs in elasmobranchs. In the latter species, glutamine synthetase is also a mitochondrial enzyme in liver and provides the substrate for carbamyl phosphate synthetase-III, an *N*-acetyl-L-glutamate–dependent, glutamine-utilizing mitochondrial enzyme [C. A. Casey and P. M. Anderson, *J. Biol. Chem.* **257**, 8449 (1982)]. It was therefore necessary to establish that the carbamyl phosphate synthetase in tortoise liver was enzyme I and not enzyme III. When glutamine was substituted for ammonia in the colorimetric assay (*19*), only 6.8 percent of the ammonia-dependent activity was obtained with the Texas tortoise. With a similar assay that contained [$^{14}$C]bicarbonate and one in which the acid-stable counts were used as criterion for citrulline formation, 10.9 percent of the ammonia-dependent activity was obtained when glutamine was substituted for ammonia with the desert tortoise. In the latter case, the reaction mixture was determined to have trace amounts of ammonia (0.4 to 0.6 m*M*). The activity was adenosine triphosphate (ATP)– and *N*-acetyl-L-glutamate–dependent in both species. The tortoise enzyme is thus carbamyl phosphate synthetase-I.
15. J. W. Campbell, in *Comparative Animal Physiology*, C. L. Prosser, Ed. (Saunders, Philadelphia, ed. 3, 1973), pp. 279–316.
16. M. A. Devaney and S. G. Powers-Lee, *J. Biol. Chem.* **259**, 703 (1984).
17. J. W. Campbell, J. E. Vorhaben, D. D. Smith, Jr., *Am. J. Physiol.* **246**, R805 (1984).
18. J. W. Gaasbeek Janzen, W. H. Lamers, A. F. M. Moorman, A. de Graaf, J. A. Los, R. Charles, *J. Histochem. Cytochem.* **32**, 557 (1984); R. Gebhardt and D. Mecke, *EMBO J.* **2**, 567 (1983).
19. Tissue fractionation and the assay of glutamine synthetase and the marker enzymes, glutamate dehydrogenase, cytochrome oxidase, lactate dehydrogenase, and glucose 6-phosphatase were essentially as previously described (*1, 10*). The 30*g* residue was not assayed for enzyme activity. Ornithine transcarbamylase was determined as described by M. Marshall and P. P. Cohen [*J. Biol. Chem.* **247**, 1641 (1972)]. Carbamyl phosphate synthetase was assayed for in a mixture containing (in micromoles per milliliter): potassium bicarbonate, 10; ammonium chloride, 10; magnesium sulfate, 10; ATP, 5; *N*-acetyl-L-glutamate, 5; L-ornithine, 5; Hepes, 40; and phosphoenolpyruvate, 2.5. Substrates were adjusted to *p*H 7.4 to 7.5 with potassium hydroxide as required. Beef liver ornithine transcarbamylase prepared as described by Marshall and Cohen (15 units), pyruvate kinase (6 units), and oligomycin (2 μg) were also added. Citrulline formed was measured colorimetrically by the method of R. M. Archibald [*J. Biol. Chem.* **156**, 121 (1944)]. For the assay of carbamyl phosphate synthetase in the desert tortoise shown in Table 1, the ATP-generating system (phosphoenolpyruvate + pyruvate kinase) was omitted. The low recovery of activity was thus most likely due to high mitochondrial adenosinetriphosphatase that was not completely inhibited by added oligomycin. Freeze-thawing was shown to have no effect on the activities of glutamine synthetase, carbamyl phosphate synthetase, or ornithine transcarbamylase.
20. H. Towbin, T. Staehlin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
21. Liver tissue from three male desert tortoises was fractionated at the University of New Mexico-Albuquerque and the fractions frozen for transport on solid carbon dioxide to Houston for assay. We thank Tom Fritts and Randy Jennings for providing this tissue as well as the necessary laboratory facilities. A single female desert tortoise was shipped from the Sonoran Desert Museum in Tucson to Houston with permission from the state of Arizona. We thank Howard Lawler of the Desert Museum for providing this specimen. Male Texas tortoises were collected under permit 501 from the state of Texas near Laredo. We thank Kent Campbell for helping with this collection. This work was supported by grant PCM82-14901 from the National Science Foundation, Metabolic Biology Program.

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## Hypertension and Sodium Salts

Whitescarver *et al.* (*1*) suggest that "hypertension development in the Dahl S [Dahl salt-sensitive] rat may be more closely related to dietary chloride consumption than to sodium consumption." In drawing this conclusion, the authors overlooked several animal and human studies assessing the effects of potassium chloride on blood pressure.

In the Dahl S rat (*2*), spontaneously hypertensive rats (*3*), and albino rats subjected to ligature of the contralateral kidney (Grollman's operation) (*4*), the addition of potassium chloride to diets containing high levels of sodium chloride mitigates the expected rise in blood pressure. Similarly, the addition of potassium chloride to diets containing "usual" (*5*) or low sodium (*6*) levels causes a decrease in blood pressure in mild hypertensives (*5*) and in normotensives with a family history of hypertension (*6*).

If chloride were the only electrolyte to affect blood pressure, the addition of potassium chloride to these animal or human diets would, by Whitescarver's theory, raise, not lower, blood pressure. Alternatively, it is possible that chloride does raise blood pressure, but to a far lesser extent than potassium lowers blood pressure, so that the net effect of administering potassium chloride would be to lower blood pressure. MacGregor observed an average decrease of 7 mmHg in systolic blood pressure when he added 60 mmol of KCl to the usual diets of 23 mild hypertensives for 4 weeks (*5*) and an average increase of 10 mmHg in systolic blood pressure after adding 100 mmol of NaCl to low sodium diets consumed by 23 mild hypertensives for 4 weeks (*7*). In these experiments, each 100 mmol of KCl lowered blood pressure an average 12 mmHg, and each 100 mmol of NaCl raised systolic blood pressure an average 13 mmHg. If chloride, not sodium, were the pressor component of salt, potassium would have to be roughly twice as potent in lowering blood pressure as chloride is in raising it.

Whitescarver *et al.* conclude that "the development of hypertension in the Dahl S rat is dependent on the provision of sodium as sodium chloride." This conclusion could only be reached if sodium chloride had been compared with a num-

ber of sodium salts, such as sodium citrate, sodium nitrite, sodium phosphate, or sodium benzoate. To date, the authors have only shown that a mixture of sodium bicarbonate and sodium amino acids does not cause hypertension in the Dahl S rat. On the basis of these findings and those of Kurtz and Morris in their study on uninephrectomized rats treated with deoxycorticosterone (*8*), one could also conclude that sodium raises blood pressure, but that this effect is canceled out by a hypothetical depressor effect of the bicarbonate ion.

We have no problem accepting the results of the two studies. We point out, however, that the effect that they demonstrate may be difficult to reproduce regularly. When Abernethy and one of us (H.G.L.) demonstrated that pressure elevation from sodium bicarbonate-acetate mixtures caused less of an increase in blood pressure than sodium chloride, the rats also gained less weight. When pair feeding was done so that there was no difference in the gain in weight, there was no difference in the gain in blood pressure (*9*). For the moment, we think that the safest interpretation of these studies is that the use of an anion other than chloride in experimental hypertension in the rat may, in some yet-to-be defined circumstance, be able to block the blood pressure-raising effects of sodium, probably by decreasing tubular reabsorption of sodium.

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**References**

1. S. A. Whitescarver *et al.*, *Science* **223**, 1430 (1984).
2. L. K. Dahl *et al.*, *J. Exp. Med.* **136**, 318 (1972).
3. W. J. Louis *et al.*, *Lancet* **1971-I**, 1283 (1971).
4. I. Bach *et al.*, *Acta Physiol.* **10**, 437 (1956).
5. G. A. MacGregor *et al.*, *Lancet* **1982-II**, 567 (1982).
6. P. S. Parfrey *et al.*, *ibid.* **1981-I**, 113 (1981).
7. G. A. MacGregor *et al.*, *ibid.* **1982-I**, 351 (1982).
8. T. W. Kurtz and R. C. Morris, Jr., *Science* **222**, 1139 (1983).
9. J. D. Abernethy *et al.*, *Fed. Proc.* **43**, 648A, Abst. 2124 (1984).

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Liebman and Langford (*1*) raise several different issues, not all of which are directly related to our study of Dahl salt-sensitive (Dahl S) rats fed diets containing high concentrations of sodium, with or without chloride (*2*).

We previously reported that dietary sodium loading with anions other than chloride (either bicarbonate alone or a combination of anions including bicarbonate, phosphate, glycinate, glutamate and aspartate—referred to as NaAA) did not produce hypertension in the Dahl S rat (*2, 3*). We have recently extended these observations to another model of salt-sensitive hypertension—uninephrectomized rats treated with deoxycorticosterone acetate (DOCA) (*4*). Blood pressures were higher in rats fed sodium chloride than in rats subjected to comparable sodium loading by means of NaAA. These observations led us to conclude that the full expression of salt-sensitive hypertension in these models is not dependent on dietary sodium alone.

In developing the NaAA diet, we combined a variety of sodium compounds so that, when compared to a high sodium chloride diet, the equivalent sodium loading without chloride would not result in differences in weight gain, arterial *p*H, net sodium and potassium balance, and plasma concentrations of sodium, potassium, and ionized calcium. At the dietary intakes required, sodium citrate chelates calcium, sodium nitrate results in decreased weight gain, sodium phosphate causes diarrhea, and sodium benzoate is a gastrointestinal irritant. After considerable trial and error, we arrived at the NaAA diet. To determine if lower blood pressures in the NaAA-treated animals might be related to a nonspecific effect of the NaAA diet itself, we studied Sprague-Dawley rats using the "one-kidney, one-clip" model of hypertension (one kidney removed and partial occlusion of the renal artery of the remaining kidney). Groups of these rats received either a high sodium chloride diet or a high NaAA diet (*4*). Over a 17-day period, both diets caused comparable increases in blood pressure. We therefore conclude that in the Dahl S rat and the DOCA-salt hypertensive rat the absence of hypertension in NaAA fed animals is specifically related to the lower intake of dietary chloride rather than to some other effect of the NaAA diet itself.

We have also evaluated the effects of selective chloride loading (without sodium) on the development of hypertension in the Dahl S rat (*4*). Blood pressure increased gradually in animals fed 4 percent sodium chloride but did not increase in animals fed equivalent amounts of chloride provided as glycine chloride. We therefore conclude that in this model the development of hypertension is dependent on the concomitant administration of sodium and chloride.

Our studies are not in conflict with the observations of others that a high dietary potassium intake tends to lower blood pressure, an effect generally attributed to potassium, not chloride. Our observations have implications for the mechanism by which sodium chloride produces hypertension in susceptible hosts.

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**References**

1. B. F. Liebman and H. G. Langford, *Science* **228**, 351 (1985).
2. S. A. Whitescarver, C. E. Ott, B. Jackson, G. Guthrie, T. A. Kotchen, *ibid.* **223**, 1430 (1984).
3. T. A. Kotchen, R. G. Luke, C. E. Ott, J. H. Galla, *Ann. Int. Med.* **98**, 817 (1983).
4. J. C. Passmore, S. A. Whitescarver, C. E. Ott, T. A. Kotchen, paper presented at 38th Annual Fall Conference of AHA Council for High Blood Pressure Research, Cleveland, Ohio, October 1984 (to be published in *Hypertension*).

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Liebman and Langford (*1*) imply that we, and others, hold that chloride is *the* pressor component of sodium chloride in the diet. In fact, we proposed the possibility that the anionic component of the sodium salt consumed can be *a* critical pathogenetic determinant of "sodium-dependent" hypertension. Given our finding that, in rats given deoxycorticosterone (DOC), provision of dietary sodium as sodium chloride induced hypertension whereas provision of dietary sodium as sodium bicarbonate did not, we concluded that it seemed prudent to speak of sodium chloride–dependent hypertension rather than "sodium-dependent" hypertension. We suggested that the pathogenesis of the DOC model of hypertension and other models of "sodium-dependent" hypertension might depend on the chloride component of sodium chloride.

In one sense, Liebman and Langford appear to agree with our suggestion that the anion of a dietary sodium salt can determine the extent to which that sodium salt induces an increase in blood pressure. They "feel that the safest interpretation of these studies [ours (*2, 3*), and those of Whitescarver *et al.* (*4, 5*] is that the use of an anion other than chloride in experimental hypertension in the rat may, in some yet-to-be defined circumstance, be able to block the blood pressure-raising effects of sodium, probably by decreasing tubular reabsorption of sodium." This formulation, however, assumes that the pressor effect of a sodium salt resides with the sodium ion alone and is critically dependent on its increased renal reabsorption.

In presupposing that sodium is *the*

pressor component of sodium chloride, and that chloride merely permits the kidney to reabsorb more sodium than it otherwise would, Liebman and Langford do not appear to allow for the possibility that dietary chloride might induce hypertension through a mechanism other than the renal retention of sodium. It is of course possible that the anion of a dietary sodium salt could affect blood pressure by affecting only the renal reabsorption of sodium. However, in rats given DOC and in Dahl salt-sensitive rats, the finding that dietary sodium chloride induces hypertension, but equimolar amounts of nonchloride containing sodium salts do not, could not be related to more positive external balances of sodium or to greater weight gain in the rats given sodium chloride (*2–5*). These observations do not support the notion that anions other than chloride block the blood pressure-raising effects of sodium simply by decreasing tubular reabsorption of sodium. The fact that provision of supplemental potassium chloride can attenuate the severity of hypertension does not exclude the possibility that some effect of chloride contributes to sodium chloride–induced hypertension.

Liebman and Langford state that it may be difficult to reproduce the finding that the anionic component of the sodium salt consumed can be a determinant of blood pressure. This finding, however, has been consistently observed in studies of the DOC model of hypertension in three separate laboratories (*2, 3, 5, 6*). The phenomenon occurs in the absence of impairment of weight gain (*2, 3, 5*).

Studies conducted thus far suggest that all sodium salts do not have the same potential to induce increases in blood pressure (*2–8*). If dietary sodium salts were shown to vary widely in their hypertensinogenic capacities in the human, the finding would have considerable relevance to the pathogenesis of "sodium-dependent" hypertension.

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**References**

1. B. F. Liebman and H. G. Langford, *Science* **228**, 351 (1985).
2. T. Kurtz and R. C. Morris, *ibid.* **222**, 1139 (1983).
3. _____, *Life Sci.*, in press.
4. S. A. Whitescarver, C. E. Ott, B. A. Jackson, G. P. Guthrie, T. A. Kotchen, *Science* **223**, 1430 (1984).
5. J. C. Passmore, S. A. Whitescarver, C. E. Ott, T. A. Kotchen, *Hypertension* **6**, 790 (1984).
6. S. M. Friedman, C. L. Friedman, J. R. Polley, *Am. J. Physiol.* **153**, 226 (1948).
7. T. O. Morgan, *Clin. Sci.* **63**, 407s (1982).
8. F. Husted *et al.*, *J. Clin. Invest.* **56**, 414 (1975).

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## Electron Density Distribution in the Organic Superconductor $(TMTSF)_2AsF_6$: Fact and Fancy

In a recent report, Wudl *et al.* (*1*) claim to have obtained experimental evidence from x-ray analysis for bonding electron density between selenium atoms belonging to different molecules of tetramethyltetraselenafulvalene (TMTSF) in the organic metal $(TMTSF)_2AsF_6$. The crystal structure is built from stacks of TMTSF molecules and sheets of $AsF_6$ anions. The evidence for bonding density consists of various features seen in an electron-density difference map. Peaks occurring between molecules of the same TMTSF stack are considered to be "a microscopic view of electron density distribution along a one-dimensional conduction band," whereas peaks between selenium atoms belonging to different stacks show that "there is a continuum of density from one molecule to the other that may represent a conduction band" (*1*, p. 417). Apart from the question why peaks in the difference map (regions of excess electron density) should be considered to represent a conduction band associated with removal of electrons from the neutral TMTSF molecules, the claims put forward in the *Science* report (*1*) are so remarkable that they can hardly avoid drawing specially critical attention to the evidence on which they are based.

A careful evaluation of the evidence seems all the more necessary because the high proportion of heavy elements in the crystal implies that bonding effects will be very small compared with the sum of the core densities and hence very difficult to detect experimentally. Indeed, the suitability factor of the compound $(TMTSF)_2AsF_6$ for a charge-density study, as defined by Stevens and Coppens (*2*), is only 0.11, compared with about 3 to 4 for a light-atom structure. A value as low as 0.11 requires extreme accuracy in the x-ray measurements and in the parameters describing the promolecule (*3*), if the difference map is to mean anything at all (*4*).

Unfortunately, the full experimental details of the work have not yet been disclosed (*5*). Yet, from the limited information provided, there is no indication of any claims for extraordinary accuracy. In fact, the main conclusions are drawn from difference maps based on room-temperature data containing only (*1*, p. 416) "minimal high-angle scattering, presumably due to a 1/3–2/3 rotational disorder observed in the $AsF_6$ groups." Although low-temperature (−113°C) measurements were also made, the resulting difference maps were considered to be inferior to those from the room-temperature study for reasons that appear to depend on the authors' a priori judgment about what the residual electron density ought to look like: "some common sense is required in the interpretation of what is an artifact of the experiment and what is 'real' electron density" (*1*, p. 417).

We are left with the internal evidence of the electron-density difference maps, which constitute figures 1 through 4 of the report (*1*). Of these, figure 1, showing the difference density in the plane of the TMTSF molecule, is the most revealing for an assessment of the quality of the maps. Although the site symmetry of the TMTSF molecule in the crystal is only $C_1$, its electron density can be expected to correspond closely to the $D_{2h}$ molecular point group. In fact, density features corresponding to chemically equivalent parts of the molecule are of quite different shapes, sizes, and strengths; for example, one selenium atom is accompanied by a strong peak in the molecular plane, in the $sp^2$ lone-pair region, but the other three selenium atoms lack this feature. It is also apparent that all the atomic centers are in regions of strong negative density, pointing to a slight error in the scale factor. Since the integral of the difference density over the unit cell must vanish, this negative density must necessarily be compensated by positive density in other regions of the crystal. Thus, many features of the difference density in the molecular plane, where it can be checked to some extent, do not seem particularly meaningful and are almost certainly due to errors in the measurements and in the parameters. It therefore seems prudent to reserve judgment about the significance of features of the difference map in the other regions where no such checks are possible.

The importance of accurate charge-density maps for studying chemical bonding can hardly be denied, but at the same time the experimental difficulties involved in obtaining such maps, particularly for heavy-atom compounds, should not be underrated. The experi-