pop might be expected to be less productive than Nal-Tel or Chapalote, and its increased prominence deserves an explanation. A plausible one is that, although the ears are small, the stalks may have been prolific, normally bearing more than one ear. The present-day race to which it bears some resemblance and to which it may be related is prolific, usually producing two or three ears per stalk.

Minor categories include cobs and kernels which appear in later levels and which are recognized as belonging to several of the modern races of Mexico described by Wellhausen *et al.* (*5*). They occur much too infrequently to be of significance in the total picture of food production but they are important in showing that these modern Mexican races were already in existence in prehistoric times. The only previous evidence of this was the fact that casts of ears appear on Zapotec funerary urns.

Other Parts of the Corn Plant

In all, 3597 specimens of parts of the corn plant, other than cobs, were found in the five caves. These specimens confirm the conclusions reached from the study of the cobs. There has been no change in the basic botanical characteristics of the corn plant during domestication. Then, as now, corn was a monoecious annual bearing its male and female spikelets separately, the former predominating in the terminal inflorescences and the latter in the lateral inflorescences, which, as in modern corn, were enclosed in husks. Then, as now, the spikelets were borne in pairs; in the staminate spikelets one member of the pair was sessile, the other pediceled. The only real changes in more than 5000 years of evolution under domestication have been changes in the size of the parts and in productiveness.

The importance of these changes to the rise of the American cultures and civilizations would be difficult to overestimate. There is more foodstuff in a single grain of some modern varieties of corn than there was in an entire ear of the Tehuacán wild corn. A wild grass with tiny ears—a species scarcely more promising as a food plant than some of the weedy grasses of our gardens and lawns—has, through a combination of circumstances, many of them perhaps fortuitous, evolved into the most productive of the cereals, becoming the basic food plant not only of the pre-Columbian cultures and civilizations of this hemisphere but also of the majority of modern ones, including our own.

Summary

Remains of prehistoric corn, including all parts of the plant, have been uncovered from fire caves in the Valley of Tehuacán in southern Mexico. The earliest remains, dated 5200 to 3400 B.C., are almost certainly those of wild corn. Later remains include cultivated corn and reveal a distinct evolutionary sequence which gave rise ultimately to several still-existing Mexican races. Despite a spectacular increase in size and productiveness under domestication, which helped make corn the basic food plant of the pre-Columbian cultures and civilizations of America, there has been no substantial change in 7000 years in the fundamental botanical characteristics of the corn plant.

References and Notes

1. P. C. Mangelsdorf and R. G. Reeves, "The origin of Indian corn and its relatives," *Texas Agr. Expt. Sta. Bull. No. 574* (1939).
2. ——— and C. E. Smith, Jr., in *Botanical Museum Leaflets, Harvard Univ.* **13**, 213 (1949).
3. P. C. Mangelsdorf, *Science* **128**, 1313 (1958).
4. ———, R. S. MacNeish, W. C. Galinat, in *Botanical Museum Leaflets, Harvard Univ.* **17**, 125 (1956).
5. E. J. Wellhausen, L. M. Roberts, E. Hernandez, in collaboration with P. C. Mangelsdorf, *Races of Maize in Mexico* (Bussey Institution, Harvard University, Cambridge, Mass., 1952).
6. R. S. MacNeish, *Trans. Am. Phil. Soc.* **48**, pt. 6, 1 (1958).
7. C. Lumholtz, *Unknown Mexico* (Scribner, New York, 1902).
8. P. C. Mangelsdorf and R. H. Lister, in *Botanical Museum Leaflets, Harvard Univ.* **17**, 151 (1956).
9. E. S. Barghoorn, M. K. Wolfe, K. H. Clisby, in *Botanical Museum Leaflets, Harvard Univ.* **16**, 229 (1954).
10. E. B. Kurz, J. L. Liverman, H. Tucker, *Bull. Torrey Botan. Club* **87**, 85 (1960).
11. E. Anderson and R. O. Erickson, *Proc. Natl. Acad. Sci. U.S.* **27**, 436 (1941).
12. The archeological excavations were supported by a grant from the National Science Foundation; the botanical studies, by a grant from the Rockefeller Foundation. Both grants are acknowledged with appreciation and thanks.

Crystalline Deamino-Oxytocin

The biological activities of this potent analog of oxytocin appear to be related to size of ring.

Derek Jarvis and Vincent du Vigneaud

When the structure of oxytocin, a hormone produced by the posterior pituitary gland and isolated from it in highly purified form (*1, 2*), was being investigated, evidence was obtained that the hormone contained a disulfide ring (*3*). That the backbone of this ring consisted of 20 members was established by the total synthesis of oxytocin (*4*) according to the structure (Fig. 1) postulated from studies in this laboratory on degradation of the hormone (*4, 5*). An identical structure was independently postulated by Tuppy (*6*).

The occurrence of a 20-membered disulfide ring in this octapeptide amide structure for oxytocin represented the first time that such a disulfide ring had been encountered in nature. Subsequently, a disulfide ring of the same size was shown to be present in insulin (*7*). A 20-membered disulfide ring was also found to occur in 8-arginine-vasopressin (*8, 9*), 8-lysine-vasopressin (*8, 10*), 8-arginine-vasotocin (*11*), and isotocin (4-serine-8-isoleucine-oxytocin) (*12*).

With the establishment of the presence of this novel structural feature in oxytocin, studies were initiated to evaluate its importance to the manifestation of some of the biological activities characteristic of this hormone. Oxy-

Dr. du Vigneaud is professor of biochemistry at Cornell University Medical College, New York City, where Dr. Jarvis was a research associate until he recently became associated with the department of pharmacology, Washington University School of Medicine, St. Louis, Mo.

tocin causes contractions of the uterus (the oxytocic effect) and brings about the release of milk in mammals (the milk-let-down or milk-ejecting effect). It also lowers the blood pressure of birds. This avian depressor effect is, in fact, the basis of the official method of assay for oxytocin in the United States Pharmacopeia. Oxytocin also has slight but definite action in raising the blood pressure and decreasing the urine flow in mammals—these being referred to as the pressor and antidiuretic effects.

That the cyclic moiety of oxytocin could not, alone, account for its pharmacological activity was shown by Ressler in this laboratory (*13*). This was accomplished by the synthesis and study of a cyclic pentapeptide amide in which the tripeptide amide side chain of oxytocin was replaced by an amide group. This cyclic pentapeptide amide had no detectable avian depressor effect and showed only slight oxytocic and milk-ejecting activities.

An indication that the biological properties of oxytocin might be related to the size of the disulfide ring was afforded by the pharmacological behavior of synthetic 4-isoglutamine-oxytocin (*14*). This substitution of isoglutamine for glutamine in oxytocin leads to an increase in the size of the disulfide ring from 20 to 22 members. However, the carboxamide grouping in the 4 position of this isomeric octapeptide is attached directly to the ring, whereas in oxytocin the carboxamide group at this position is separated from the ring by two methylene units. Neither oxytocic nor avian depressor activity was detected in the 4-isoglutamine-oxytocin. 5-Isoasparagine-oxytocin, an analog possessing a 21-membered ring, with the carboxamide group at the 5 position attached directly to the ring, was also found to be inactive (*15*).

Evidence that the relationship to the ring of the carboxamide group of the asparagine residue has great influence on the activity of oxytocin resulted from the study of 5-glutamine-oxytocin (*16*), 4-asparagine-oxytocin (*17*), and 4-asparagine-5-glutamine-oxytocin (*17*). Only the 4-asparagine-oxytocin showed appreciable activity. Moreover, studies of 4-decarboxamido-oxytocin (4-α-aminobutyric acid-oxytocin) (*18*) and 5-decarboxamido-oxytocin (5-alanine-oxytocin) (*18*) have shown the vital importance of the presence of the carboxamide group of asparagine to the activity of the hormone, since the 5-decarboxamido-oxytocin is practically inactive and 4-decarboxamido-oxytocin is fairly potent. Thus, the lack of activity of isoglutamine-oxytocin and isoasparagine-oxytocin is, in all probability, not attributable to the change in ring size alone.

1-(Hemi-homocystine)-oxytocin, the analog of oxytocin which is obtained by substituting a hemi-homocystine residue for the hemicystine residue of the hormone which bears the free amino group, is almost completely devoid of biological activity (*19*). The substitution in this instance leads simultaneously to an increase, by one methylene unit, in the size of the ring and in the separation of the amino group from the disulfide bond. Analogs of oxytocin studied thus far, in which there is an additional amino acid residue in the ring, enlarging it to 23 members, were virtually inactive (*20*).

Studies centered around a highly active analog of oxytocin, deamino-oxytocin (*21, 22*), have now provided significant data on the importance of ring size to biological activities characteristic of oxytocin. The structure of this analog, lacking the free amino group but having a ring of the same size as that in oxytocin, is shown in Fig. 2.

Highly purified, amorphous deamino-oxytocin exhibited the principal pharmacological properties of oxytocin to a very high degree (*22, 23*). It was found to possess an avian depressor activity of 733 $\pm$ 23 units per milligram, approximately 50 percent higher than that of oxytocin. Its oxytocic activity, tested with isolated uterine strips of rats in natural estrus, was 551 $\pm$ 17 in comparison with 546 $\pm$ 18 units per milligram for oxytocin under the same conditions (*24*). It also exhibited a milk-ejecting activity about the same as that of oxytocin. The antidiuretic activity was about fivefold higher than that of oxytocin, whereas the rat pressor activity was only about one-third that of oxytocin.

The high potency of deamino-oxytocin (1-β-mercaptopropionic acid-oxytocin) provided an opportunity to study more directly the importance of ring size to pharmacological activity by adding a "CH_2" unit to, or subtracting a "CH_2" unit from, the β-mercaptopropionic acid residue in position 1. This was accomplished by synthesizing 1-γ-mercaptobutyric acid-oxytocin and 1-mercaptoacetic acid-oxytocin, which contain a 21-membered ring and a 19-membered ring, respectively. The methods of synthesis and purification paralleled those used for deamino-oxytocin. In the case of 1-γ-mercaptobutyric acid-oxytocin, the protected intermedi-

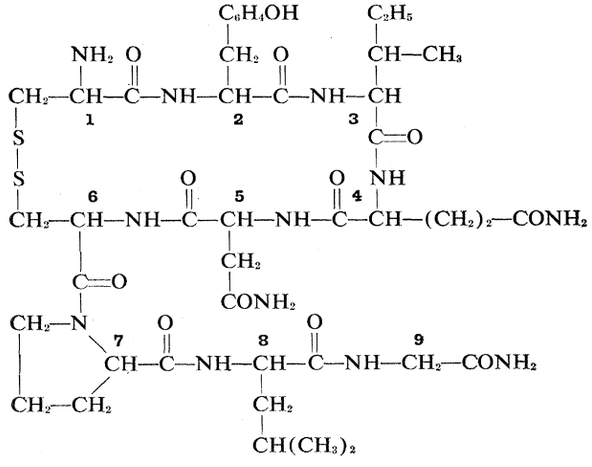

Fig. 1 (left). Oxytocin. the structure.

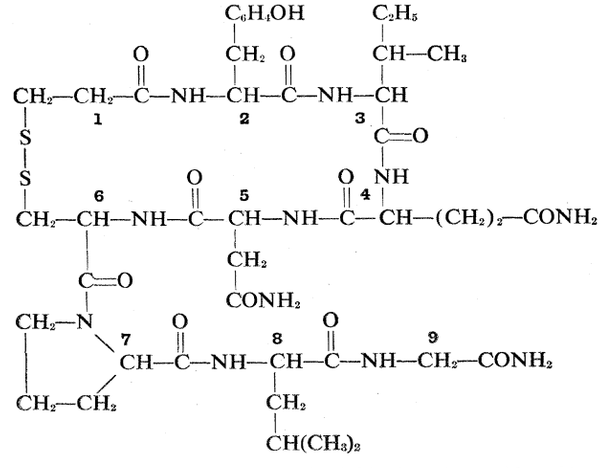

Fig. 2 (right). Deamino-oxytocin. The numbers indicate the position of individual amino acid residues in

ate S-benzyl-γ-mercaptobutyryl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucyl-glycinamide was synthesized and reduced with sodium in liquid ammonia. The resulting disulfhydryl compound, 1-γ-mercaptobutyric acid-oxytoceine, was oxidized to the corresponding cyclic disulfide in aqueous solution by means of potassium ferricyanide, and purified by countercurrent distribution. The desired compound was obtained as a white, fluffy powder possessing satisfactory elemental and amino acid analyses. This analog was found to be almost devoid of avian depressor and oxytocic activities. 1-Mercaptoacetic acid-oxytocin was prepared from the appropriate intermediate and purified in an almost identical manner, and the purified material gave the expected analytical values. This analog was found to posses 4 units per milligram of avian depressor activity and 25 units per milligram of oxytocic activity. Thus, it can be clearly seen that the high pharmacological activities possessed by deamino-oxytocin are greatly reduced when one "CH_2" unit is deleted, and are almost completely eliminated when an additional "CH_2" unit is introduced. These striking alterations in pharamacological properties may be due to changes in the relationship of the side chains of the constituent amino acids to each other and to the disulfide bond, such changes having been induced by the changes in ring size.

Alternatively, deamino-oxytocin may be looked upon as an analog of 1-mercaptoacetic acid-oxytocin to which activity has been restored, in high degree, simply by the insertion of a "CH_2" unit into the residue in the 1 position. Hence it is conceivable that the insertion into 1-mercaptoacetic acid-oxytocin of a "CH_2" unit at points other than the 1 position might also restore appreciable activity. As one step in this direction we have synthesized 1-mercaptoacetic acid-6-(hemi-homocystine)-oxytocin, a homolog of 1-mercaptoacetic acid-oxytocin in which a "CH_2" unit has been inserted in the 6 position. This compound possesses a ring of the same size as that in deamino-oxytocin and differs from deamino-oxytocin only with regard to the distribution of methylene units about the disulfide bond. This 1-mercaptoacetic acid-6-(hemi-homocystine)-oxytocin exhibited 16 units per milligram of oxytocic activity and no detectable avian depressor activity. Thus, in these structures possessing rings of the same size, the shifting of a "CH_2" unit from one side of the disulfide bond to the other affects the molecule in such a way as to cause a profound change in the pharmacological behavior.

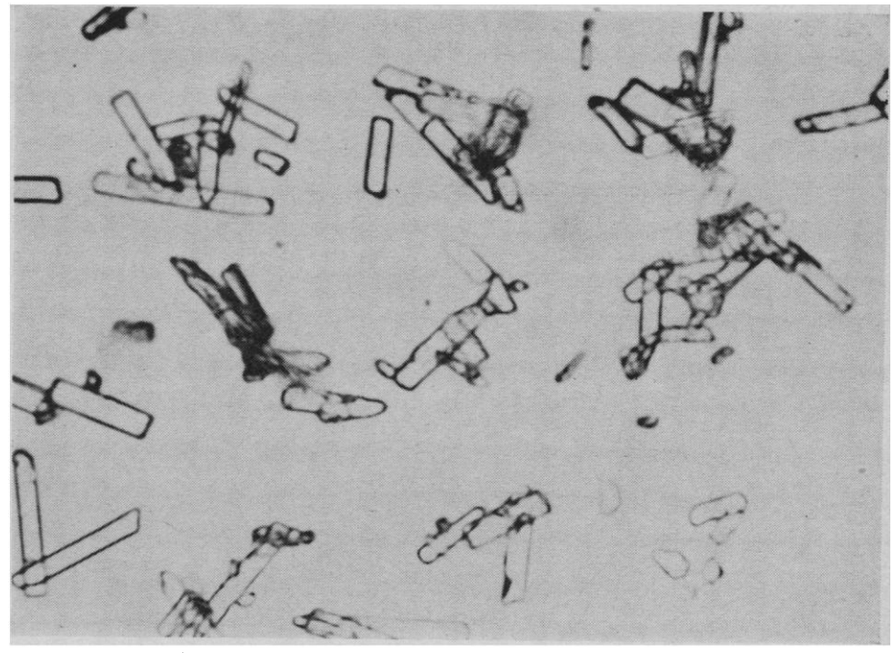

Fig. 3. Crystals of deamino-oxytocin.

When an attempt was made to dissolve the highly purified amorphous 1-γ-mercaptobutyric acid-oxytocin in a small amount of 1*N* acetic acid, the analog unexpectedly crystallized. The amorphous compound had dissolved almost completely in the 1*N* acetic acid and this solution, in the presence of a slight amount of the remaining solid, had been allowed to stand at room temperature. After several hours it was noticed that crystals were beginning to form. A quantitative experiment was then performed. One hundred milligrams of the lyophilized powder (moisture content approximately 8 percent) was completely dissolved in 4 ml of acetic acid. The solution was diluted with 20 ml of water, seeded and left to stand for 8 hours at room temperature, in which time the analog separated as thin hexagonal plates (66 mg).

The crystallization of 1-γ-mercaptobutyric acid-oxytocin is the first instance of the crystallization of an analog of oxytocin insofar as we know. It might be pointed out that although crystalline salts of oxytocin with flavianic acid (*2*) and *p*-hydroxyazobenzene-*p'*-sulfonic acid (*25*) have been described, the free hormone itself has not, as yet, been crystallized.

The fortuitous crystallization of 1-γ-mercaptobutyric acid-oxytocin immediately suggested the exciting possibility that deamino-oxytocin might be induced to crystallize under similar conditions since, as already mentioned, the structures of the two compounds differ only by one methylene unit. Approximately 20 mg of deamino-oxytocin in the form of a lyophilized powder (*22*) was dissolved in 1 ml of 1*N* acetic acid. To this solution an additional small amount of solid was added; the mixture was shaken to wet the solid and then left to stand overnight at room temperature. By the following morning a heavy deposit of crystals (Fig. 3) had formed. The crystalline deamino-oxytocin was filtered off and was found to possess high avian depressor and oxytocic activities. Another sample (70 mg; moisture content 7 percent) of lyophilized powder was dissolved in 2.5 ml of 1*N* acetic acid, seeded and left to stand at room temperature whereupon crystallization took place. The crystals (30 mg) were filtered off and dried at room temperature under reduced pressure for 10 hours. The deamino-oxytocin dried in this manner had an avian depressor activity of approximately 900 units per milligram and an oxytocic activity of approximately 750 units per milligram on isolated uterine strips of rats in natural estrus, higher values than had been found previously for the amorphous material (*22–24*).

When the sample was heated at 100°C under reduced pressure over P_2O_5 for 5½ hours, there was a loss in weight of 1.7 percent. This heated sample was then assayed. No decrease in biological activity was detected as a result of the heating. If crystalline deamino-oxytocin after suitable study meets other requirements for stability, it may merit consideration for possible use as an international standard for the assay of oxytocin. Intensive pharmacological studies would be in order to explore this possibility, if criteria of stability for this purpose are met.

The crystallization of these two closely related analogs, deamino-oxytocin (1-β-mercaptopropionic acid-oxytocin) and 1-γ-mercaptobutyric acid-oxytocin, one highly active and the other practically inactive, affords for the first time crystalline analogs of oxytocin for use in various physical and physical-chemical studies. The availability of these two analogs may possibly facilitate the elucidation of the relationship between molecular architecture and biological activities which are characteristic of oxytocin.

References and Notes

1. A. H. Livermore and V. du Vigneaud, *J. Biol. Chem.* **180**, 365 (1949).
2. J. G. Pierce, S. Gordon, V. du Vigneaud, *ibid.* **199**, 929 (1952).
3. J. M. Mueller, J. G. Pierce, H. Davoll, V. du Vigneaud, *ibid.* **191**, 309 (1951); R. A. Turner, J. G. Pierce, V. du Vigneaud, *ibid.* **193**, 359 (1951).
4. V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis, S. Gordon, *J. Am. Chem. Soc.* **75**, 4879 (1953); V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis, *ibid.* **76**, 3115 (1954).
5. C. Ressler, S. Trippett, V. du Vigneaud, *J. Biol. Chem.* **204**, 861 (1953).
6. H. Tuppy, *Biochim. Biophys. Acta* **11**, 449 1953); see also H. Tuppy and H. Michl, *Monatsh. Chem.* **84**, 1011 (1953).
7. F. Sanger, L. F. Smith, R. Kitai, *Biochem. J.* **58**, vi (1954); A. P. Ryle, F. Sanger, L. F. Smith, R. Kitai, *ibid.* **60**, 541 (1955).
8. V. du Vigneaud, H. C. Lawler, E. A. Popenoe, *J. Am. Chem. Soc.* **75**, 4880 (1953).
9. V. du Vigneaud, D. T. Gish, P. G. Katsoyannis, *ibid.* **76**, 4751 (1954); P. G. Katsoyannis, D. T. Gish, V. du Vigneaud, *ibid.* **79**, 4516 (1957); V. du Vigneaud, D. T. Gish, P. G. Katsoyannis, G. P. Hess, *ibid.* **80**, 3355 (1958).
10. V. du Vigneaud, M. F. Bartlett, A. Jöhl, *ibid.* **79**, 5572 (1957).
11. P. G. Katsoyannis and V. du Vigneaud, *J. Biol. Chem.* **233**, 1352 (1958); *Nature* **184**, 1465 (1959).
12. R. Acher, J. Chauvet, M. T. Chauvet, D. Crepy, *Biochim. Biophys. Acta* **58**, 624 (1962); S. Guttmann, B. Berde, E. Stürmer, *Experientia* **18**, 445 (1962); A. Jöhl, A. Hartmann, H. Rink, *Biochim. Biophys. Acta* **69**, 193 (1963).
13. C. Ressler, *Proc. Soc. Exptl. Biol. Med.* **92**, 725 (1956).
14. ——— and V. du Vigneaud, *J. Am. Chem. Soc.* **79**, 4511 (1957); C. Ressler and J. R. Rachele, *Proc. Soc. Exptl. Biol. Med.* **98**, 170 (1958).
15. W. B. Lutz, C. Ressler, D. E. Nettleton, Jr., V. du Vigneaud, *J. Am. Chem. Soc.* **81**, 167 (1959).
16. R. A. Boissonnas, S. Guttmann, P.-A. Jaquenoud, J.-P. Waller, *Helv. Chim. Acta* **39**, 1421 (1956).
17. P.-A. Jaquenoud and R. A. Boissonnas, *ibid.* **45**, 1601 (1962).
18. V. du Vigneaud, G. S. Denning, Jr., S. Drabarek, W. Y. Chan, *J. Biol. Chem.* **238**, PC1560 (1963); *ibid.* **239**, 472 (1964).
19. D. Jarvis, M. Bodanszky, V. du Vigneaud, *J. Am. Chem. Soc.* **83**, 4780 (1961).
20. S. Guttmann, P.-A. Jaquenoud, R. A. Boissonnas, H. Konzett, B. Berde, *Naturwissenschaften* **44**, 632 (1957); R. A. Boissonnas, S. Guttmann, B. Berde, H. Konzett, *Experientia* **17**, 377 (1961).
21. V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, R. D. Kimbrough, Jr., *J. Biol. Chem.* **235**, PC64 (1960). In this and the following reference the deamino-oxytocin is referred to as desamino-oxytocin. In a subsequent paper in *J. Biol. Chem.* (*18*) the name deamino-oxytocin was adopted at the request of the editors.
22. D. B. Hope, V. V. S. Murti, V. du Vigneaud, *ibid.* **237**, 1563 (1962).
23. W. Y. Chan and V. du Vigneaud, *Endocrinology* **71**, 977 (1962).
24. W. Y. Chan, M. O'Connell, S. R. Pomeroy, *ibid.* **72**, 279 (1963).
25. M. Bodanszky and V. du Vigneaud, *Nature* **184**, 981 (1959).

26. This work was supported in part by grant HE-01675 from the National Heart Institute. The bioassays were carried out under the direction of W. Y. Chan of this laboratory, to whom we express our appreciation.

News and Comment

Appropriations: The Critics of Congress Often Slight an Inner Redoubt of the System

The literature of analysis and abuse of the U.S. Congress is a rich one, but for a year or two it has been evident that criticism of the national legislature has been mounting toward one of its periodic peaks.

Liberal critics argue that Congress is unresponsive to the exigencies of the latter half of the 20th century. Resistance to innovating legislation they attribute to rigid rules and customs which they claim make Congress, in practice, an extremely undemocratic institution. Concentrated fire has been turned on the Senate filibuster and the House Rules Committee, and broader criticism is directed at a feudalistic committee system, which, fused to the principle of seniority, makes the inheritance of power in Congress depend primarily on survival.

While study of congressional pathology is popular these days, relatively little attention is paid to an extremely significant area of action—the domain of the appropriations committees in the House and Senate. Because appropriations business is so complicated and is usually conducted so discreetly in committee and so smoothly on the floor, the public is likely to be little aware of its import. But just as a connoisseur of football often watches the line play rather than the backfield, an observer of Congress can instructively focus on the appropriations process.

It is true that Representative Otto Passman (D–La.), chairman of the House appropriations subcommittee which deals with foreign aid appropriations, in recent years has gained public notice by organizing an annual Donnybrook over the aid program's appropriations. It is also true that, with much less spectacle, Albert Thomas (D–Tex.), as chairman of two appropriations subcommittees which control the funds for the space program and the National Science Foundation, exercises a strong influence over the federal science effort.

It is gratefully acknowledged within the medical research community, also, that Representative John Fogarty (D–R.I.) and Senator Lister Hill (D–Ala.) have used their appropriations subcommittee chairmanships to enhance the fortunes of the great federal health research program. But the system which gives these men their leverage seems to get little attention.

The appropriations committees derive their influence from a main well of congressional authority, the right to spend or withhold money. Federal programs which involve the spending of money require a dual legislative process—authorization and appropriation. Standing legislative committees