Table 1. Cell-free protein synthesis with brain polysomes from animals exposed to dark and light. Incubation was at 37°C for 30 minutes in 0.2 ml containing 5 μmole of tris-Cl, 0.3 mg of ribosomal protein, 0.6 mg of supernatant protein, 2.0 μmole of adenosinetriphosphate, 2.0 μmole of Mg^{++}, 2.0 μmole of phosphoenolpyruvate, 0.5 μmole of guanosinetriphosphate, 10 μg of phosphoenolpyruvate kinase, 20 μmole of NH_4Cl, .02 μmole each of 19 unlabeled amino acids, and 0.25 μc of leucine C^{14} (168 μc/μmole). Portions (0.1 ml) of the reaction mixture were transferred to filter paper disks and processed according to the technique of Mans and Novelli (*14*). The counting efficiency was 40 percent. Each value represents an average of three determinations.

Conditions	Radioactivity (count/min per mg protein)
Complete, light-exposed ribosomes	3010
Complete, dark-exposed ribosomes	1470
Minus ribosomes	80
Light-exposed ribosomes minus supernatant	33
Dark-exposed ribosomes minus supernatant	72
Complete, light-exposed ribosomes plus RNAase	104
Complete, dark-exposed ribosomes plus RNAase	190

thesis in vivo as well as in vitro. This hypothesis is also supported by the fact that decreased amino acid incorporation into protein in the brains of older rats in vivo and in vitro is associated with smaller populations of polysomes (and consequently less functioning messenger RNA) (*8, 10*).

Previous investigations have characterized polyribosomes by their sedimentation behavior in sucrose density gradients, their appearance in the electron microscope, their susceptibility to digestion by ribonuclease, their resistance to digestion by deoxyribonuclease and proteolytic enzymes, and their ability to incorporate labeled amino acid into polypeptide chains. The brain polysomes isolated from young rats possess characteristics which differ only slightly from those of liver preparations. Heavier sucrose is required for resolution of brain ribosomes, possibly because larger aggregates are present and the peptides have, on the average, greater molecular weights. Also, concentrations of ribonuclease greater than those used in similar experiments on liver ribosomes are needed for digestion (5 μg compared to 0.5 μg).

It is not clear from our experiments how environmental stimuli were translated into macromolecular changes. It is tempting to assume that the local release of neurotransmitter from a presynaptic terminal results in postsynaptic macromolecular changes, analogous to the effect of hydrocortisone on the liver (*11*). However, no data are available on this matter; and it is equally plausible that circulating hormones or other factors mediate the response in brain but not in liver.

The response of brain messenger RNA to environmental stimulation not necessarily correlated with "learning" that can be assayed raises an interesting problem. If stimulation alone may evoke this important macromolecular response, we must question the specificity of the participation of RNA in the memory process as has been suggested by the experiments of Zemp *et al.* (*12*) and Hyden (*13*). Our data suggest that template RNA's are involved in any enhanced participation of neural cells within a communal response, but that increases or decreases in messenger RNA may not, per se, specify the extent and duration of the neural information storage.

S. H. APPEL
Departments of Medicine and *Neurology, Hospital of University of Pennsylvania, Philadelphia*

W. DAVIS
S. SCOTT
Departments of Medicine and *Anatomy, Duke University, Durham, North Carolina*

References and Notes

1. H. Hyden and H. Hartelius, *Acta Psychiat. Neurol. Scand.*, Kbh Supp. **48** (1948); S. Brattgard, *Acta Radiol. Supp.* **96** (1952).
2. H. Hyden and E. Egyhazi, *Proc. Nat. Acad. Sci. U.S.* **48**, 1366 (1962).
3. G. P. Talwar, S. P. Chopra, B. K. Goel, B. D'Monte, *J. Neurochem.* **13**, 109 (1966).
4. S. H. Barondes and M. W. Nirenberg, *Science* **138**, 813 (1962); T. Staehelin, F. O. Wettstein, H. Noll, *ibid.* **140**, 180 (1963); J. R. Warner and A. Rich, *J. Mol. Biol.* **10**, 202 (1964).
5. While this manuscript was in preparation a detailed characterization of brain polysomes, including their electron microscopic and sedimentation characteristics, appeared (A. T. Campagnoni and H. R. Mahler, *Biochemistry* **6**, 956 1967).
6. We thank Dr. Walter Guild for assistance in performing the sedimentation analysis of the single ribosome peak.
7. H. Noll, T. Staehelin, F. O. Wettstein, *Nature* **198**, 632 (1963).
8. S. H. Appel, unpublished observation.
9. ———, *Nature* **213**, 1253 (1967).
10. R. V. Murthy, *Biochim. Biophys. Acta* **119**, 599 (1966).
11. G. M. Tomkins and E. S. Maxwell, *Annu. Rev. Biochem.* **32**, 677 (1963).
12. J. W. Zemp, J. E. Wilson, K. Schlesinger, W. O. Boggan, E. Glassman, *Proc. Nat. Acad. Sci. U.S.* **55**, 1423 (1966).
13. H. Hyden, *Prog. Nucleic Acid. Res.* **6**, 187 (1967).
14. R. J. Mans and B. D. Novelli, *Arch. Biochem. Biophys.* **94**, 48 (1961).
15. Supported in part by PHS grants GM 516-06 and NB-06283 and by a NIH career development award to S.H.A. We thank Drs. M. Moses and J. B. Wyngaarden for their helpful discussion.

19 June 1967

Drinking Induced by Carbachol: Thirst Circuit or Ventricular Modification?

Levitt and Fisher (*1*) reported that carbachol applied to a variety of subcortical-limbic sites caused the animal to drink. If carbachol was applied to any one of these sites and atropine to any other such site, drinking elicited by application of carbachol was reduced. Control tests showed that application of sodium chloride at one site did not reduce drinking evoked by application of carbachol. Since the reduction of carbachol-induced drinking caused by application of atropine at any two subcortical sites was always reciprocal, these results may support their view that the "neural basis for the thirst drive consists of complex alternative and reciprocal pathways of neurons susceptible to activation by cholinergic stimulation." I now suggest an alternative to the Levitt–Fisher view which may, in addition, be of some value in interpreting certain data not clearly understood at present.

In a recent experiment (*2*) we stimulated the caudate nucleus of rats, and noticed that three of ten treated rats consistently drank more water than controls during a 10-minute observation period. In cases where the animals drank, probes tended to locate in medial caudate regions (Fig. 1). Why then did chemical stimulation of the caudate nucleus induce drinking? One possible answer is that stimulation is, in fact, affecting the adjacent ventricle. In a recent report on drinking induced by application of carbachol (*3*) it was noted that histological results "indicate a localization of carbachol-induced responding close to the midline, with effectiveness falling off with increasing distance laterally. . . . This large region of the hypothalamus affected by carbachol does not coincide with any obvious anatomical structure." Part of the drinking effect induced by the application of carbachol may be mediated through the ventricle, and such drinking may be caused by modification of the milieu of the ventricle, or by stimulation of receptors that may line the wall of the ventricle, or by both (*4*). The results of Levitt and Fisher, while possibly interpretable in their view on the basis of redundant neural circuitry, might alternatively be understood within the framework of the present hypothesis. Thus, whatever drinking mechanism carbachol activates by way of

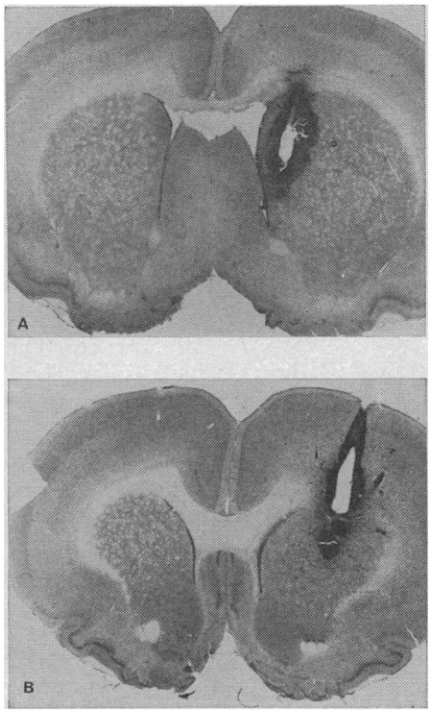

Fig. 1. Sections taken at the caudate nucleus (A) in which the animal consistently drank when the brain was stimulated with carbachol, and (B) in which the animal never drank when carbachol was applied. Marked pathological changes resulted from repeated crystalline and liquid chemical injection. Cresyl violet, × 10.

the ventricle may be blocked by atropine. The latter drug would exert its influence by way of the ventricular system as well.

Related experiments, in which the brain was stimulated chemically (*5*), are somewhat difficult to understand in terms of present anatomical and physiological concepts. First, carbachol applied to fornix axons causes the animal to drink. Since it is presumed that carbachol is acting at synaptic sites, it is not clear how carbachol is achieving its result. Second, chemical stimulation of septal area causes the animal to drink; septal lesions produce the same effect (*6*). How is drinking augmented by both stimulating and destroying the same tissue? Third, in the experiments of Levitt and Fisher and Fisher and Coury (*5*) it is not apparent why the thirst system should be so widely represented throughout the limbic system. Finally, the areas indicated by Grossman that produce drinking caused by application of carbachol are typically medial and dorsal to the hypothalamic areas that cause adipsia when lesioned (*7*).

The proposal that drinking caused by stimulation of the brain with carbachol may, at times, be brought about by ventricular modification offers a possible resolution to these issues. First, such a view is helpful in understanding why stimulation of the fornix with carbachol produces drinking. Thus, the present view would suggest that carbachol diffuses from the fornix to the ventricle and then exerts its effect. Second, the increased drinking caused both by chemical stimulation of the septal area and by electrocoagulative lesions may be explained by assuming that stimulation effects are mediated by the ventricle while lesion effects are mediated by the destruction of nervous tissue.

Another possibility is that both the lesion and the carbachol stimulation are affecting the ventricle in such a way that the animal drinks more. Third, the wide distribution of the "thirst circuit" might be understood in terms of the wide distribution of the third and lateral ventricles in subcortical areas. Finally, the disparity in anatomical localization between points producing drinking by application of carbachol and points that modify drinking by lesions might similarly be understood as a difference in site of action.

If this suggestion is valid, certain conclusions are possible. First, a distinction between direct and indirect effects resulting from chemical stimulation of the brain may prove useful. When the caudate nucleus is stimulated with carbachol the animal turns contralaterally; an indirect effect of the drug is increased drinking by the animal. It is possible that drinking induced by the administration of carbachol may have both direct and indirect actions in certain hypothalamic regions, while only indirect ventricular action when applied elsewhere. Second, it would be of interest to discern the mechanisms for this drinking which is presumed to be induced by ventricular action. The paraventricular nucleus may be important in this regard since it is excited by acetylcholine and increases its activity with increased thirst (*4*). Finally, several at present unresolved problems concerning stimulation of the brain with chemicals might be resolved if the locus of action and extent of diffusion were determined.

ARYEH ROUTTENBERG
Department of Psychology,
Northwestern University,
Evanston, Illinois

References and Notes

1. R. A. Levitt and A. E. Fisher, *Science* **154**, 520 (1966).
2. A. Routtenberg and R. Stiles, in preparation.
3. A. J. Feider, thesis, Indiana University, 1965.
4. C. G. Smoller, *Science* **147**, 882 (1965); C. McC. Brooks, T. Ishikawa, K. Koizumi, H-H. Lu, *J. Physiol. London* **182**, 217 (1966).
5. S. P. Grossman, *Science* **132**, 301 (1960); A. E. Fisher and J. N. Coury, *ibid.* **138**, 691 (1962); N. E. Miller, *ibid.* **148**, 328 (1965).
6. J. A. Harvey and H. F. Hunt, *J. Comp. Physiol. Psychol.* **59**, 49 (1965).
7. B. K. Anand, *Physiol. Rev.* **41**, 677 (1961).
8. Supported by PHS contract MH 11991-02.

20 February 1967

The issue raised by Routtenberg has been covered in some detail in the proceedings of a recent symposium, particularly in Fisher's paper and reply to Gloor (*1*). The points made by Gloor and by Routtenberg are compelling, and we agree with the general premise that workers using chemical brain stimulation need be more concerned about diffusion patterns of specific chemicals used in their studies. It is of some significance that even Miller's excellent review of the field (*2*) contains no mention of the diffusion problem or of the possibility of ventricular transport after the chemicals are injected into the brain. The long delay in coming to grips with this issue probably derives from the extent to which positive results in early studies were confined to single sites or zones, and from the immensity of the task involved in obtaining accurate information about diffusion gradients for each chemical at each brain site studied.

In reference to Routtenberg's hypothesis, there are three additional lines of evidence that should be advanced in its favor: (i) drinking has not as yet been successfully triggered in rats by electrical stimulation of most limbic sites where a cholinergic stimulus has proven effective. Even though many chemicals may be more selective in neural action than electrical stimulation, the effects of the two techniques should show a higher positive correlation than has been demonstrated. (ii) The minimum drinking latency after localized brain injection of a cholinergic substance is about 3 minutes, and much longer latencies frequently occur. It is easier to postulate diffusion to a distant target site than to explain a prolonged delay in effective neurotransmitter action at the site of injection. (iii) The fact that atropine blocks cholinergically induced drinking when applied to a positive *contralateral* site is most difficult to interpret unless one assumes that diffusion may be influencing the results.

Thus, the indirect evidence for a diffusion or ventricular-involvement hypothesis seems relatively strong. Individual points could be countered, many of them convincingly, but we would have to agree at this time that a demonstration of ventricular involvement would clarify much unruly data. Nevertheless, it is difficult to find direct evidence in favor of the ventricular-involvement hypothesis. There seem to be almost no data in the literature concerning the ease or difficulty of diffusion through brain into intact ventricular spaces, particularly when crystalline substances are used. Diffusion presents a greater problem after liquids are injected, yet Myers (*3*) finds no evidence of ventricular involvement after injection of small volumes of dyes (0.5 to 2 μl) into brain tissue. Dyes of 229 to 960 molecular weight diffused through an average of 1 mm^3 in 25 minutes after 0.5-μl injections. Pilot work in our laboratory indicates that even less diffusion occurs when crystalline stains are used. Also, a number of workers in the field have provided evidence against the proposal that positive results correlate highly with closeness of the injection site to a ventricular space (*4*). In our own studies, we often obtain negative results 0.25 to 0.5 mm from a ventricle and positive results 1.5 to 2.0 mm distant from one. Under the circumstances, however, more must be learned about diffusion than can be discovered by measuring distances from ventricles or by injecting dyes that are chemically unrelated to the test substances. It is certain that chemicals diffuse at different rates and it is possible that local conditions at injection sites vary to the extent that a chemical injected at a distance from a ventricular space occasionally may have easier access to blood or cerebrospinal fluid than chemicals injected in the immediate vicinity of a ventricle.

What about injection into the ventricles themselves? Feldberg's studies have established the fact that many chemicals can diffuse from ventricular spaces into brain tissue, inducing a variety of behaviors (*5*). We have recently completed a study to determine whether drinking can be elicited by stimulation within a ventricle. A critical test close to midline structures is difficult since the chemical would also have direct access to relevant limbic tissue surrounding the region of ventricular puncture. Therefore, injection sites were selected in the posterior horn of the lateral ventricle [for example, 2.2, 6.0, +1, DeGroot atlas (*6*) coordinates]. The following results were obtained. Direct ventricular injection of the amount of crystalline carbachol we usually insert into brain (1 to 3 μg) produced catatonia, tremors, or bizarre motor behavior. Significant amounts of drinking did not occur. Injection of 1 part carbachol to 4 parts eserine or noradrenaline (1 to 3 μg total) into the ventricle frequently did produce a significant drinking response (8 to 17 ml within 1 hour) with latencies of 10 minutes or longer. Also, atropine or hyosine applied within the ventricle successfully blocked cholinergic induction of drinking at other brain sites. It is clear, therefore, that cholinergic substances and their blocking agents can act within, or diffuse from the ventricular spaces to influence tissue involved in the mediation of drinking behavior, and that drinking following cholinergic stimulation is a low threshold response, easily masked by the effects produced by higher concentrations of the drug.

Although such data favor Routtenberg's hypothesis, it should be made quite clear that the test is not critical. The critical question is whether microquantities of the crystalline substances used in our studies frequently diffuse through 1 to 2 mm of brain tissue, enter an intact ventricular space, and act there or diffuse back into brain within a short space of time. Our first attempts to answer this question have not produced data favoring Routtenberg's hypothesis. Thus far, sites which are close to but not within the posterior horn of the lateral ventricle have failed to yield positive results. Since the corpus callosum overlays the posterior horn, and since Feldberg reports that diffusion through white matter is often slow or negligible (*5*), the ventricle has been approached from the hippocampal side as well as from above. None of the animals so prepared have been observed to drink significant amounts following normal or reduced cholinergic stimulation. However, additional animals do need to be tested.

With reference to the difficulty of penetration of white matter by water-soluble substances, it would appear difficult for Routtenberg to explain our reports of drinking to cholinergic stimulation of cingulate cortex (*7*). The thickest portion of the corpus callosum overlays access to ventricular spaces in this region, and if diffusion into subarachnoid spaces were involved, other cortical areas should prove equally positive.

It is doubtful, however, that definitive answers can be obtained with these techniques. It will be necessary to inject radio-isotopes of key chemicals into brain tissue and perfuse from ventricular spaces at selected time intervals after the injection. Such experiments, however, will only establish if and when certain quantities of tagged material diffuse into ventricles. Further tests will be necessary to establish whether the chemicals are acting at one or many loci. The clearest test of the concept of a thirst-mediating system of cholinergically sensitive neurons might come from studies combining cholinergic stimulation with electrical recording techniques. Evidence on specific changes in firing patterns of single or multiple neurons in positive versus negative cholinergic drinking sites after the brain has been stimulated with cholinergic substances or with blocking agents should help settle the question. In this regard, it is relevant that Wayner (*8*) reports that systemic injection of hypertonic saline produces increased unit firing in the same structures that our laboratory has implicated in chemically induced drinking and eating (*7*, *9*). Many other studies, including some using electrical stimulation, attest to the fact that neurons mediating behavior related to hunger, thirst, sex, and sleep are very widely represented throughout the limbic system (*10*). Whatever the reasons for this wide distribution of primary drive substrates which Routtenberg questions, the phenomenon seems well documented.

The key question at issue, however, is whether complex, functionally discrete neural matrices use a specific chemical transmitter throughout their trajectory, and whether the results of our mapping studies have been influenced markedly by diffusion into ventricles. It does seem clear that scientists using chemical brain stimulation techniques will need to consider ventricular involvement among the growing list of factors requiring careful analysis and control. If diffusion through the ventricles should prove to be a major contaminating variable, it will become necessary to establish thresholds of response after stimulation of the ventricles as opposed to stimulation of the brain tissue. If a neural site responds to a lower level of chemical stimulation than a nearby ventricular site, the possibility of ventricular involvement in the me-

diation of the response would seem to be eliminated. Dosages as low as 0.05 μg of carbachol have produced drinking when applied to brain tissue. Equivalent data on injection into ventricles are not yet available.

ALAN E. FISHER
ROBERT A. LEVITT
Department of Psychology, University of Pittsburgh, Pittsburgh, Pennsylvania

References and Notes

1. A. E. Fisher, in *Neural Regulation of Food and Water Intake, Ann. N.Y. Acad. Sci.*, in press.
2. N. E. Miller, *Science* **148**, 328 (1965).
3. R. D. Myers, *Physiol. Behav.* **1**, 171 (1966).
4. For example, S. P. Grossman, *Amer. J. Physiol.* **202**, 872 (1962); J. N. Coury, thesis, Univ. of Pittsburgh (1967).
5. W. Feldberg, *A Pharmacological Approach to the Brain from Its Inner and Outer Surface* (Williams and Wilkins, Baltimore, 1963).
6. J. DeGroot, *Verhandel. Koninkl. Ned. Akad. Wetenschap. Afdel. Natuurk.* **II**, 52 (1959).
7. A. E. Fisher and J. N. Coury, *Science* **138**, 691 (1962); in *Thirst*, M. J. Wayner, Ed. (Pergamon, New York, 1964).
8. M. J. Wayner, in *Thirst*, M. J. Wayner, Ed. (Pergamon, New York, 1964); in *Neural Regulation of Food and Water Intake, Ann. N.Y. Acad. Sci.*, in press.
9. J. N. Coury, *Science* **156**, 1763 (1967).
10. P. D. MacLean and D. W. Ploog, *J. Neurophysiol.* **25**, 29 (1962); B. W. Robinson and M. Mishkin, *Science* **136**, 260 (1962); R. Hernandez-Peon, G. Chavez-Ibarra, P. J. Mogane, C. Timo-Taria, *Exp. Neurol.* **8**, 93 (1963).
11. Supported by NIMH grant MH-1951 to A.E.F.

12 July 1967

Extrusive Lunar Ring Structures?

O'Keefe, Lowman, and Cameron (*1*) argue that the curious morphology —convex profile, evidence of postmare age, and patterned surface—of the slopes of the Flamsteed Ring hills indicate an extrusive flow structure. Their suggestion that the entire Flamsteed Ring is the surface expression of a ring dike would be stronger if it could be shown that the curious morphology is peculiar only to lunar craters similar to the Flamsteed Ring, and is not found in the typical, bowl-shaped craters with sharp, raised rims, widely interpreted to impact explosion pits. However, this is not the case. Figure 1 shows a number of examples of craters with sharp, raised rims that show the same curious convex toe at the rim base where the rim abuts the mare surface.

There are three working hypotheses: (i) neither the Flamsteed Ring nor the majority of other craters are extrusive, and the morphology discussed by O'Keefe, Lowman, and Cameron is not related to crater genesis; (ii) the Flamsteed Ring (and similar structures) is extrusive but the majority of other craters aren't; (iii) all craters' rims are formed by extrusive flow.

Those who see in the majority of lunar craters a continuous sequence of structures having a single mode of origin (hypotheses i and iii) see in the Flamsteed Ring a damaged crater nearly destroyed in the "flooding" process which formed the maria. Examples of craters partly destroyed by invading mare material abound. On the other hand, the hypothesis of O'Keefe *et al.* suggests that while most lunar craters are thought to result from impacts, the Flamsteed Ring, with its circular pattern of curiously formed hills, may be extrusive.

Figure 1 shows that the patterned ground described by O'Keefe *et al.* is common to many structures, even classic sharp-rimmed flooded craters. This observation effectively eliminates the argument of O'Keefe *et al.* for hypothesis (ii). For this reason, as well as for the reasons listed by Milton (*2*), and Goles and Taylor (*3*), hypothesis (ii) is rejected in favor of some form of mass wasting as the proper explanation of the morphology of lunar slopes. Available evidence indicates that this morphology occurs in conjunction with the mare material, suggesting that the process of emplacement of the mare material may enhance the mass wasting. The mare material is probably some form of volcanic material, judged by its association with tectonic structures, and hence moonquakes or plastic slumping due to contact heating or partial melting come to mind as agents. To my knowledge, this morphology has not been found in a non-mare environment, though the majority of photos are of mare regions. The craters in Fig. 1 can thus be interpreted as typical bowl-shaped impact craters, partly filled with mare material, and with slumped walls.

That there are no known terrestrial cases of simple, extrusive, crater-like ring structures on earth argues against hypothesis (iii). It continues to appear that lunar surface relief is a mixture of impact craters, collapse and maar-type pits (often in chains) fault and graben structures, and vast, flat lava flows. There is still no conclusive published evidence that any major positive relief structures are the result of extrusion, and the morphology noted by O'Keefe *et al.* is probably indicative of some other process.

WILLIAM K. HARTMANN
Lunar and Planetary Laboratory, University of Arizona, Tucson

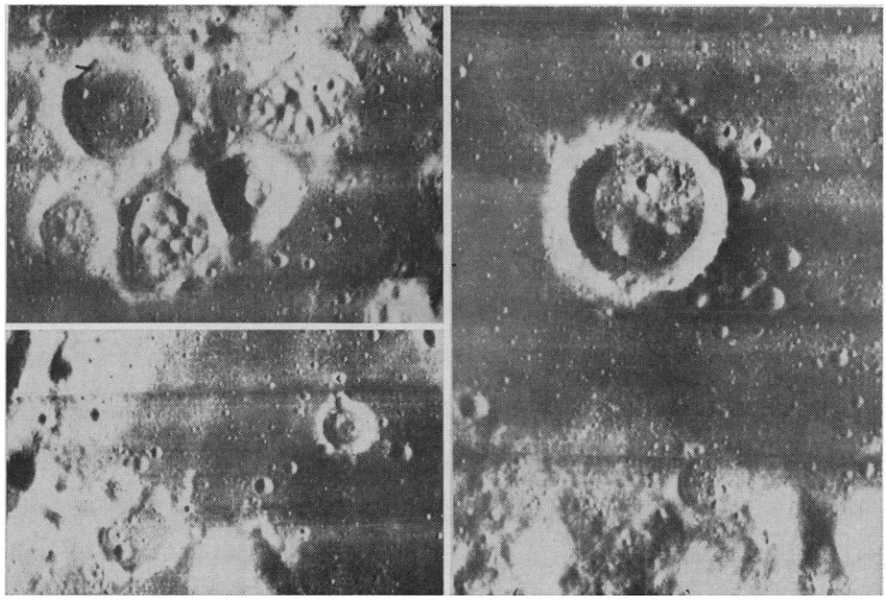

Fig. 1. Examples of craters showing peculiar convex toe at the bottom of wall slopes. Although partly flooded by mare material, many of these have well-preserved, sharp, raised rims characteristic of ordinary, "fresh" lunar craters. [Photo, courtesy NASA]

References

1. J. A. O'Keefe, P. D. Lowman, Jr., W. S. Cameron, *Science* **155**, 77 (1967).
2. D. J. Milton, *ibid.* **156**, 1135 1967).
3. G. Goles and S. R. Taylor, *ibid.*, p. 1134.

20 April 1967