made in uninfected cells and labeled with [^{14}C]glucosamine. (ii) Antiserum can be used to enrich for, if not completely separate, membranes carrying distinct antigens on their surfaces.

Our results are significant from the following points of view. (i) Smooth membranes from infected cells carrying new virus-specific glycoproteins also contain viral antigens accessible to antibody. (ii) The binding of antibody to antigens on the surface of the membrane increases the density of membranes in proportion to antibody concentration. (iii) The binding of the viral antigen to the membrane—and we assume this is one or more of the viral membrane glycoproteins—is sufficiently strong to withstand hydrodynamic forces acting upon it and hence it is not due to some nonspecific adsorption. (iv) Our technique may find useful application for the demonstration of surface antigens in isolated membranes, for the isolation or enrichment of membranes containing a particular antigen, and ultimately for mapping of antigens on membrane surfaces.

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3 September 1970

Urinary Adenosine 3′,5′-Monophosphate in the Switch Process from Depression to Mania

Abstract. *Marked elevations of urinary adenosine 3′,5′-monophosphate occurred on the day of rapid switch from a depressed into a manic state in patients with manic-depressive illness. It is suggested that this increase might serve a trigger function for the process by which catecholamines are elevated during the manic phase of the illness.*

The purpose of this report is to report changes in urinary adenosine 3′,5′-monophosphate (cyclic AMP) that are associated with the "switch process" from depression to an agitated manic state. This specific psychobiological process is accompanied by a rapid and profound change in mood, behavior, and thought content which probably reflects sudden marked alterations in brain metabolism. Our previous work has suggested that an alteration in brain catecholamine metabolism may precede and accompany these behavioral changes. Our evidence suggests a sharp increase in urinary norepinephrine and a decrease in total sleep and in rapid eye movement (REM) sleep on the day before the onset of a manic episode (*1*, *2*).

We have further suggested a relationship between mood alteration and cyclic AMP excretion in patients with affective disorders; however, this work did not focus on the point of change from one mood state to another (*3*). In mania, we reported that 24-hour urinary excretion of cyclic AMP is elevated over normals, whereas in severe depression cyclic AMP excretion is significantly diminished (*3*). Abdullah and Hamadah confirmed these findings (*4*). Patients treated with L-dopa (L-dihydroxyphenylalanine) showed dose-related increases in cyclic AMP excretion in association with clinical change (*5*). A double-blind study was completed in which both manic and depressed patients were treated with lithium carbonate. Urinary cyclic AMP followed the direction of clinical change in this investigation (*5*).

Several studies suggest a possible involvement of cyclic AMP as a mediator of behavioral change. We have shown that centrally acting drugs such as L-dopa, MAO (monoamine oxidase) inhibitors, amphetamine, pentobarbital, and chlorpromazine produce changes in the levels of brain cyclic AMP in mice and rats (*6*). Furthermore, when the lipid-soluble dibutyryl derivative of cyclic AMP was injected into the lateral ventricle of cats, a syndrome of agitation and hyperexcitability resulted. However, when dibutyryl cyclic AMP was injected into the mesencephalic reticular formation, a catatonic-like state was produced (*7*).

In this study a marked transient increase in urinary cyclic AMP in association with the switch process from depression to mania was found.

Patients were hospitalized on two metabolic research wards at the National Institute of Mental Health. Immediately after admission, the diagnosis was established by a team of psychiatrists and corroborated by examination of previous hospital records and by psychological testing. The patient population included five females and one male, ranging in age from 20 to 45 years. Behavioral data were collected on a longitudinal basis. Each patient was rated every 8 hours by a trained nursing research team on items that evaluate depression and mania (*8*). The reliability of this rating scale has been consistently documented over the past 7 years. The patients' behavioral changes were further documented by the nursing staff, who dictated vignettes concerning the patients' verbal and nonverbal behavior every 8 hours. The day of onset of mania was designated as the "switch day," defined as the first day of marked increase in motor and verbal behavior. The depressive phases were characterized by retardation, seclusiveness, depressive mood, and the patients' being nonverbal; the manic states were characterized by grandiosity, continuous talking, increased motor activity, psychosis, bizarre dress and behavior, flight of ideas, and intrusiveness. Total hours of sleep were estimated by the nursing staff through half-hourly room checks.

All patients were on a constant low catecholamine and indoleamine diet throughout the time of the study. Coffee and tea were restricted to one cup per day, thus minimizing the intake of methylxanthines. Drugs were not administered to the patients included in the study during the time of investigation with the exception of one male patient who received L-dopa continuously before, during, and after the period of study.

Urine was collected in 24-hour pools throughout the period of investigation; it was refrigerated immediately, aliquoted, and frozen within 3 days. Sodium metabisulfite was added to

all samples. Urine creatinines performed on the specimens were consistent with comp'ete urine volume collections. Urinary cyclic AMP was analyzed by the enzymatic isotopic displacement method of Brooker *et al.* (*9*) as described previously (*3*).

Figure 1 shows the mean mania ratings and urinary cyclic AMP values obtained for the seven episodes in the six patients studied. The switch into mania occurred rapidly in all patients and took place over a period ranging from 2 to 24 hours. The onset of mania was characterized by the sudden appearance of constant verbalization and increased motor activity. An increase in urinary cyclic AMP was observed on the switch day into mania; the level was significantly higher than the mean of all prior days but was not significantly elevated over the days that followed the switch into mania. This increase on the switch day occurred in six of the seven episodes. In the seventh episode, there was a significant increase in cyclic AMP on the day prior to the switch. As can be seen from the mean values in Fig. 1, there was also a tendency for the excretion of cyclic AMP to increase on days -1 and -2.

In five out of seven episodes, a decrease in cyclic AMP excretion occurred after the initial increase on the switch day. In two episodes, the post-switch values during the manic episodes remained elevated over the values obtained during the depressed period.

Figure 2 gives a phenomenological description of the sequence of a patient's behavioral changes in association with concomitant alterations in cyclic AMP excretion. This represents the one case where cyclic AMP increased on the day prior to the major increases in manic symptomatology. However, there was a mild increase in symptoms on day -1.

The two patients with the most rapid onsets of mania showed the most striking elevations of cyclic AMP on the switch day. Before the switch, the first patient who showed a particularly rapid change in behavior excreted a mean level of 5 μM cyclic AMP per 24 hours. The level rose to 20 μM on the switch day and dropped on the next day to 3 μM; and concentration remained at this level despite high mania ratings and marked physical activity and agitation. The second patient excreted a mean level of 22 μM per 24 hours before the switch; this level abruptly increased to 100 μM on the switch day, then dropped the following day to 20 μM, and again remained low during the manic period. In this study we have found increases in urinary vo'ume on the switch day with concomitant significant increases in urinary cyclic AMP concentration paralleling the changes in total daily cyclic AMP excretion. The significance of the increase in cyclic AMP excretion in mania cannot be explained by volume, since there is an overall significant increase in concentration as well as total amount.

Sutherland has described cyclic AMP as a "second messenger," the synthesis of which is increased by a stimulus that may be a hormone or a neurotransmitter substance (*10*). Once produced, cyclic AMP has an intracellular triggering effect on key enzyme systems, which have functions that are specific to particular target organs.

Although an increase in levels of cyclic AMP precedes the metabolic process, a persistently high level of this nucleotide is not always necessary for the maintenance of such a process. For example, ACTH (adrenocorticotropic hormone) increases cyclic AMP levels in the adrenal gland 3 to 7 minutes after its administration and results in a corticosteroidogenic response (*11*). Although the high levels of cyclic AMP fall rapidly after 10 minutes, the corticosteroidogenic response lasts for a period of hours (*12*). Similarly, in isolated fat cells, thought to be adrenergic receptors, norepinephrine stimulates the production of cyclic AMP, which initiates the lipo'ytic process that persists for hours after the cyclic AMP levels have decreased to baseline, further indicating that the participation of cyclic AMP may function, in part, as a "trigger" mechanism (*13*).

Numerous studies have described an important relationship between the catecholamines and cyclic AMP in peripheral tissues as well as in brain. We have treated mice with L-dopa plus MAO inhibitors in order to maximize brain norepinephrine and dopamine

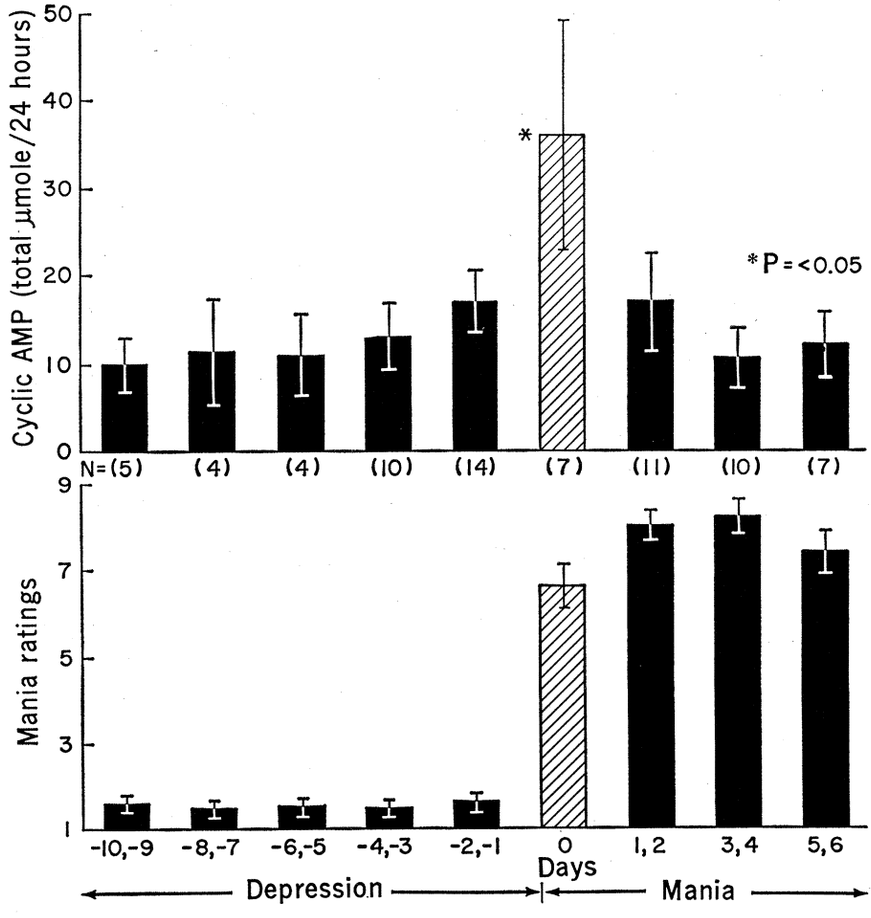

Fig. 1. Increase in urinary cyclic AMP associated with the switch from depression to mania ($N = 7$ episodes).

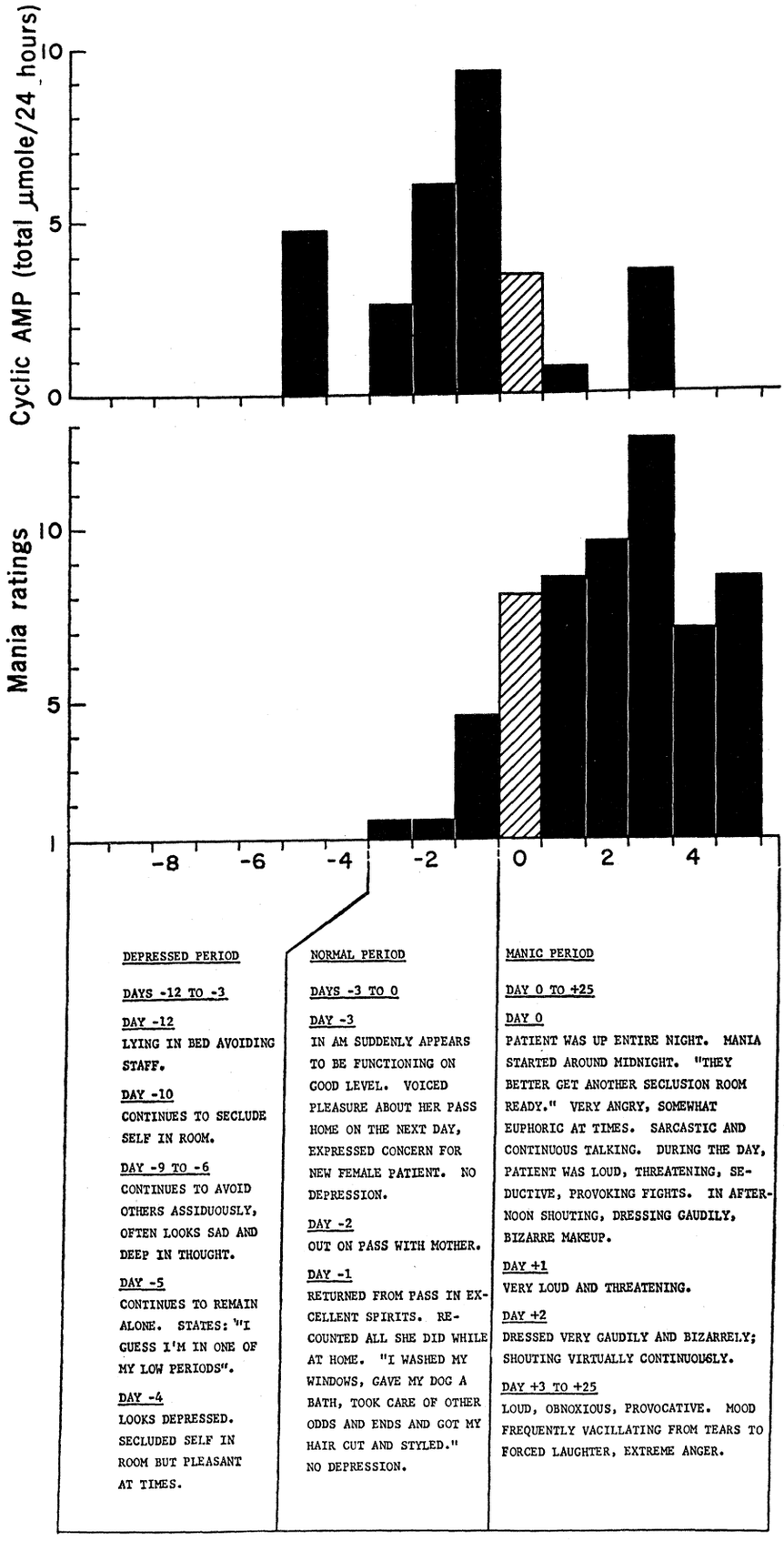

Fig. 2. Alterations in excretion of cyclic AMP, correlated with behavioral changes, in one patient during switch into mania.

and have demonstrated altered brain cyclic AMP levels in these animals. Mice treated in this manner displayed marked directed aggressiveness as well as hyperactivity (*6*). Bunney *et al.* have recently described a series of events associated with the shift from depression to mania in a series of ten patients (*1*). On the day before the onset of mania, urinary norepinephrine excretion increased sharply while total sleep time and REM time decreased. During the manic episode, urinary catecholamine excretion remained elevated and was associated with decreased sleep and REM time. The finding of elevated urinary catecholamines in mania is compatible with previous studies (*14*). Observation of a transient peak of cyclic AMP associated with a persistent elevation of urinary norepinephrine raises the possibility of a relationship between the two events. The monoamine theory of mood states that mania is associated with a functional excess of bioactive amines at central synapses (*15*) and that the converse is associated with depression (*15, 16*). Increase in excretion of catecholamines probably reflects increased synthesis rates centrally as well as peripherally. It is possible that the transitory peak in cyclic AMP may be related to the events leading to increased catecholamine excretion.

In this study, we have shown marked elevations of urinary cyclic AMP on the switch day and a trend toward an increase on days −1 and −2. In five out of seven episodes, a decrease in cyclic AMP occurred after the initial increase on the switch day. We found a subgroup of patients whose post-switch values during the manic episodes remained elevated over the values obtained during the depressed period. Although we investigated only five patients with mania at persistently high levels (*3*), we had not previously investigated the period of switch from one mood state to another. The transience of the rise and fall of cyclic AMP excretion in this study may accurately reflect the events occurring around the switch time or may be a reflection of the heterogeneity of manic-depressive illness.

In our earlier work, we have found that physical activity does not play a significant role in cyclic AMP excretion (*3*). Elevations were not associated with agitated psychotically depressed patients. The amount of cyclic AMP

excreted by ten hyperkinetic children off medication was similar to that excreted by an age-matched group of normal controls. In addition, the mean values for both the hyperactive patients and control children were in the same range as our adult normal controls. After pro'onged physical activity (football), there was no significant difference between the pre- and postexercise levels of urinary cyclic AMP in seven normal subjects. In contrast to our findings, one study suggested that exercise may elevate urinary cyclic AMP levels (*17*). Robison *et al.* also recently reported normal levels of cerebral spinal fluid cyclic AMP in manic patients; however, they did not study patients at the time of the switch into mania and thus would have missed a transient marked peak at that time (*18*). In our studies, cyclic AMP excretion is independent of age and sex (*3*).We have reviewed the known factors influencing cyclic AMP excretion (*3, 5*), but the relative proportion of cyclic AMP coming from extrarenal sources remains to be determined. Thus, at this time, one cannot state whether the cyclic AMP response is mediated centrally or is a reflection of peripheral metabolism. Decreases in the excretion of urinary cyclic AMP have been demonstrated in patients with pseudohypoparathyroidism. Thus, changes in calcium metabolism may be associated with alterations in cyclic AMP metabolism (*3*). Although the changes in urinary cyclic AMP may be secondary to catecholamine or calcium changes, our evidence documents an alteration in an important process that accompanies and, in at least one instance, preceded gross behavioral changes. This further suggests the importance of biochemical changes in the manic-depressive illness. It is of interest to consider the possibility that the concept of cyclic AMP as a trigger mechanism for metabolic processes may be relevant to the switch process from depression to mania.

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Speech Perception in Infants

Abstract. *Discrimination of synthetic speech sounds was studied in 1- and 4-month-old infants. The speech sounds varied along an acoustic dimension previously shown to cue phonemic distinctions among the voiced and voiceless stop consonants in adults. Discriminability was measured by an increase in conditioned response rate to a second speech sound after habituation to the first speech sound. Recovery from habituation was greater for a given acoustic difference when the two stimuli were from different adult phonemic categories than when they were from the same category. The discontinuity in discrimination at the region of the adult phonemic boundary was taken as evidence for categorical perception.*

In this study of speech perception, it was found that 1- and 4-month-old infants were able to discriminate the acoustic cue underlying the adult phonemic distinction between the voi ed and voiceless stop consonants /b/ and /p/. Moreover, and more important, there was a tendency in these subjects toward categorical perception: discrimination of the same physical difference was reliably better across the adult phonemic boundary than within the adult phonemic category.

Earlier research using synthetic speech sounds with adult subjects uncovered a sufficient cue for the perceived distinction in English between the voiced and voiceless forms of the stop consonants, /b-p/, /d-t/, and /g-k/, occurring in absolute initial position (*1*). The cue, which is illustrated in the spectrograms displayed in Fig. 1, is the onset of the first formant relative to the second and third formants. It is possible to construct a series of stimuli that vary continuously in the relative onset time of the first formant, and to investigate listeners' ability to identify and discriminate these sound patterns. An investigation of this nature (*2*) revealed that the perception of this cue was very nearly categorical in the sense that listeners could discriminate continuous variations in the relative onset of the first formant very little better than they could identify the sound patterns absolutely. That is, listeners could readily discriminate between the voiced and voiceless stop consonants, just as they would differentially label them, but they were virtually unable to hear intraphonemic differences, despite the fact that the acoustic variation was the same in both conditions. The most measurable indication of this categorical perception was the occurrence of a high peak of discriminability at the boundary between the voiced and voiceless stops, and a nearly chance level of discriminability among stimuli that represented acoustic variations of the same phoneme. Such categorical perception is not found with nonspeech sounds that vary continuously along physical continua such as frequency or intensity. Typically, listeners are able to discriminate many more stimuli than they are able to identify absolutely, and the dis-