OLFACTORY THRESHOLDS IN NORMAL AND ADRENALECTOMIZED RATS
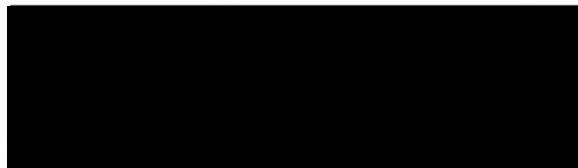
By

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INTRODUCTION

The role of hormones in neural processes is only starting to be fully understood. Evidence is accumulating to suggest that a much more extensive interaction occurs than had been previously believed (Beach, 1948). In particular, the effects of adrenal corticoids on neural tissues appear to be widespread and dramatic. Hoagland (1954) measured conduction velocity and nerve excitability in rats by applying a shock to the toe pad and measuring the resultant primary cortical response in the somatic recieving area. The latency of the cortical evoked response was taken as a measure of conduction time from toe to cortex. In anesthetized, adrenalectomized rats there was a significant slowing in conduction speed. The latter could be restored to normal by treatment with cortisone, whereas injection of a placebo had no effect. Consistent with the observed shift in conduction time was the decrease seen in the excitability of the sciatic nerve (in situ) in adrenalectomized, salt-maintained rats. Slocombe, Tozian, and Hoagland (1954) attended to certain inconsistancies reported in the literature regarding adrenocorticoid effects on nervous tissues. They noted that adrenalectomy increases excitability to seizure inducing shocks, but slows the frequency of EEG. They also reported that adrenal removal results in a slowing of conduction speed of impulses from toe to cortex but only for near-threshold shocks. Finally, responses of the gastrocnemius muscle in adrenalectomized rats not maintained on adrenal cortical extract fatigue in one-sixth the time required to fatigue those adrenalectomized animals who were on adrenal cortical extract therapy. The authors proposed that the reason for these

different effects may lie in the variety of physiological processes effected by adrenalectomy. In order to distinguish between probable intrinsic changes in excitability and effects brought about by changes in the immediate environment of the nerve, they measured the excitability of the sciatic nerve both in situ and in vitro. This shift could not be overcome by bathing the nerve in properly balanced electrolyte solution or by an atmosphere of O_2. However, injecting adrenal cortical extract partially restored excitability within 3 hours. Apparently, the observed changes were due to some intrinsic effect of steroids on the nerve. Woodbury (1954) has shown that brain excitability, as measured by electroshock seizure threshold, is increased by the presence of corticosteroids, and Henkin, Gill, and Bartter (1963) have demonstrated an increase in peripheral axonal conduction velocity in untreated patients with adrenal insufficiency. Yet conduction across the myoneural junction was significantly delayed. These shifts in conduction were not modified by the administration of Na-K active steroids but did return to normal with glucocorticoid hormone therapy. Endroczi (1969) suggests that in the forebrain and brainstem structures excitability changes may be the primary effect of corticoid hormones.

Relating changes in brain function to alterations in steroid levels, Ojeman and Henkin (1967) recorded evoked potentials for visual stimuli in adrenocortical insufficient patients under diverse conditions of steroid replacement. A significant lengthening in latency for each wave of the evoked response was seen in untreated subjects, and this latency was decreased after treatment with glucocorticoids, but not following desoxy-corticosterone acetate administration. Similar data have been reported for

cats (Feldman, 1962). Here, evoked response latencies from sciatic nerve to thalamus were found to be significantly lengthened in adrenalectomized cats.

Sensory processes also include some as yet unspecified mechanism of interaction between neural tissues and adrenal glucocorticoids. Henkin (1970) found dramatic changes in the sensitivities for taste, smell and hearing in adrenal insufficient humans. Olfactory detection thresholds for solutions of NaCl, KCl, $NaHCO_3$, sucrose, urea, and HCl in those subjects were found to be lower by factors of 1000 to 10,000,000 than those in normals (Henkin & Bartter, 1966). Further, the sensitivities could be returned to normal in 12 to 24 hours following pregnenolone or any other glucocorticoid treatment. Terminating the administration was followed in 5 to 7 days by a significant decrease in thresholds. Treatment with desoxy-corticosterone acetate did not alter olfactory sensitivity. Of interest is the fact that patients with nonadrenal pathologies but who had developed severe hyponatremia and contraction of extracellular fluid volume displayed normal odor sensitivity. In patients with cystic fibrosis, despite having normal serum concentrations for Na^+ and K^+, olfactory thresholds were as low as those for adrenal insufficient patients. Treatment with desoxycorticosterone acetate or pregnenolone, with accompanying changes in water and electrolyte metabolism, did not alter sensitivity. Apparently, olfactory sensitivity is independent of changes in serum concentrations of Na^+ and K^+ and shifts in extracellular fluid volumes.

There is also a significant increase in taste sensitivity in adrenal insufficient patients for all taste modalities (Henkin & Solomon, 1962).

Treatment with desoxycorticosterone acetate for 2 to 9 days does not effect the thresholds, but the administration of glucocorticoids restores thresholds to normal within 18 to 36 hours.

In hearing, a similar phenomenon was found (Henkin, McGlone, Daly, & Bartter, 1967). The auditory threshold curve for adrenal insufficient patients not on steroid therapy was below that of normals but could be normalized by glucocorticoid therapy. Again, the administration of desoxy-corticosterone acetate had no effect on sensitivity, although it resulted in a decrease in serum K^+ concentration and an increase in body weight. In patients with panhypopituitarism, treatment with ACTH over 4 days returned the auditory thresholds to normal.

The influence of adrenal steroids on sensory thresholds has not been clearly demonstrated for subhuman species. In the case of olfactory sensi-tivity, only two papers have been published, both utilizing adrenal insuf-ficient patients, reporting these threshold shifts. Henkin, et al. (1966) evaluated smell sensitivity by presenting a narrow-necked, 60-ml glass or 30-ml plastic bottle containing the odorant solution, 2 to 5 cm beneath the nose with the mouth closed. Each test solution was presented to the patient together with two blank or carrier solutions and the subject was asked to specify which of the three solutions smelled different from the other two.

Blast olfactometry was employed by Pruszewicz and Kosowicz (1966), that is, a known quantity of odorant substance under a determined gas flow and pressure was presented directly to the subjects nares during a short period of apnea.

In the sniff technique used by Henkin, et al., the detection thresholds were specified in terms of the concentration of the odorant solution in the bottle, which at best was only a relative and unstable estimate of the concentration of odorant molecules in the gas phase above the solution. The actual concentration of the latter is affected by the solution temperature and concentration, the partial pressure of the odorant and the air turbulence between the subject's nose and the top of the bottles at any particular time. Similar problems are associated with the blast technique except here the interference of proprioceptive stimulation of nasal tissues by the air stream is more likely. In both instances, therefore, a rigorous quantification of the olfactory shifts had not been achieved.

The present investigation explored the generality of the threshold shift phenomenon in the olfactory modality. More importantly, an attempt was made to rigorously control all relevant parameters of the olfactory situation in order to establish the magnitude and limits of these changes.

METHODS

Normal and adrenalectomized male rats were trained to bar press for water reinforcement in the presence of deodorized air and to suppress this behavior in the presence of air containing the odor of pyridine. After the subjects were found to be satisfactorily discriminating the two stimulus conditions, the intensity of the pyridine odor was reduced, in random magnitudes, until threshold intensities were fixed. ADX subjects were then administered corticosterone and/or placebo and thresholds were again determined.

Subjects

Six naive hooded male rats of the Long-Evans strain were used as subjects. At approximately 40 days of age two animals, and at 90 days of age one animal, were bilaterally adrenalectomized using the dorsal approach under ether anesthesia. One more adult subject was adrenalectomized following the determination of his threshold for pyridine. All subjects were at least 100 days old when the experiment was formally begun. The animals were motivated by depriving them of water for from 20 to approximately 23.5 hours before each experimental session. Following each session, at the beginning of the experiment, each subject was given an amount of water which was sufficient to maintain his body weight at 80% of ad libitum weight (as determined at 100 days of age). However, as the experiment progressed and the animals became more efficient at the discrimination, their weights were progressively increased so that prior to the determination of thresholds for pyridine they weighed between 100 and 115% of their ad libitum weight. The ADX subjects

received a solution of 1% saline and 5% dextrose, both as a reinforcement and for maintenance, and intact subjects received tap water. The subject that was adrenalectomized at 90 days of age, although immediately postoperatively was found to be devoid of plasma adrenal corticoid, was eventually capable of secreting normal amounts of corticosterone. It was assumed that a regeneration of vital adrenal tissues had occurred.[1] Therefore, hereafter this subject will be included in the intact group. Purina rat chow was constantly available in the individual home cages.

Apparatus

The apparatus used in this study incorporates those features of an olfactometer which have been assessed to be of critical importance in the control of test atmosphere and stimulus presentation (Pfaffmann, Goff, & Bare, 1958; Johnston, 1967; Ough & Stone, 1961; Wenzel, 1948). The response chamber (Figure 1) was made of stainless steel plating, 12-in. long and 4-in. square in cross section, open at each end. At the forward end, on what would constitute the right side of the chamber, a dipper mechanism was installed such that a water dipper could be presented to the subject without interfering with air flow or contaminating air purity. A negative pressure outside the chamber assured the flow of air out of the dipper receiving hole. A horizontal bar projected from the middle of the floor, at the front of the enclosure, and six 1/8-in. vertical stainless steel

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[1]Failure to completely adrenalectomize a subject may be due either to a rupture of the adrenal gland during removal, leaving a small amount of steroid-producing tissue in the body cavity, or to the presence of accessory nodules of adrenal corticoid-secreting tissues. (Richter, 1941; Hahn, 1969)

rods set 1/2 in. apart and installed forward of the bar confined the subject within the chamber. At the rear of the enclosure a rectangular cap, also containing vertical stainless steel rods, was put into position after the animal was introduced to the response chamber. This cap was attached to a 4-in. diameter flexible, accordion-type hose. The opposite end of the hose was attached to a venting hood and, thus, provided efficient active evacuation of air introduced into the chamber from the front. Periodic tests were conducted to assure that air flows were in the appropriate directions, that is, that the chamber was being properly exhausted by the action of the hood, yet air flowed out of the test chamber at the receiving hole. A speaker was connected to the external surface at the front end of the chamber to provide continuous background noise. A glass sleeve which could be easily removed for cleaning following each subject's daily session was designed to fit inside the stainless steel chamber. The entire bar-mechanism-dipper-mechanism-chamber arrangement was housed in a refrigerator shell and was mounted on a waterproofed wooden frame so that it could be removed and appropriately cleaned and dried.

The achievement and control of air purity was realized through the incorporation of an elaborate olfactometer (Figure 2). Two air systems were utilized, one to provide the steady stream of deodorized air, and a second to facilitate the presentation of odorous molecules. A T arrangement at an outlet valve on the laboratory's compressed air line provided two air supplies. Each of these were led through a National Cylinder Gas regulating valve to a 20-liter carboy reservoir which served to dampen any significant oscillations in air pressure within the lines. From these tanks, air passed to a series

of three gas diffusion bottles containing Purafil[2], calcium chloride, and a layered combination of charcoal and silica gel, respectively. Each system then passed through a coil to a flowmeter. From this point, the deodorized background air, moving at approximately 40L/min., entered a manifold where it was fractionated into six equal streams. Each of the six lines then led to a glass T.

The air stream to be odorized, moving at approximately 1L/min., also entered a 6-outlet manifold. Each of the output streams from the manifold led to a microregulating valve, then to a gas diffusion bottle. The air was odorized by sparging through the odorant solution. The odorous air was then directed to a three-way stopcock. The normally open (flowing) pathway of each stopcock led to a ventilating hood by way of a six-way reducing manifold. Thus, a continuous air flow through each solution bottle was assured, and variability of odor concentrations within the gas was avoided. A flowmeter was used to monitor the rate of flow out of the individual stopcocks. On the downstream (normally closed) side of each stopcock a glass T was connected (the same T to which the deodorized background air was flowing). The third arm of the T (output) was directed, by 1/4-in. O.D. teflon tubing, through a wall of the refrigerator shell to a pyramidal-shaped, stainless steel diffusion chamber. Finally, the latter was latched to the animal chamber when a subject was to be run.

The sparging bottles and the coils were immersed in a constant temperature bath which contained distilled water recirculated through a charcoal-glasswool filter. Each bottle and stopcock was removed at the end of the

[2]Purafil is a registered trademark of the Borg-Warner Corporation. (Hanna, Kuehner, Karnes, & Garbowicz, 1964)

day, washed and dried. Odorant solutions were freshly prepared just prior to the start of each subject's session and put into a particular predetermined bottle, However, the concentration of the solution for a particular channel was randomly alternated every other session. Four channels contained odorant solutions and two had deodorized (blank) solutions. By operating a particular stopcock, the experimenter could add a specific concentration of odorant to the background air stream. Pyridine was used as the olfactory stimulus for all testing, and its intensity was varied by diluting concentrated pyridine with distilled, deodorized water. All parts of the apparatus which were between the reservoirs and the diffusion chamber were made of Pyrex glass, teflon, or 316 stainless steel and were easily disassembled for cleaning.

Procedure

All subjects were first trained to bar press for water reinforcement on a continuous reinforcement (CRF) schedule. Then, they were introduced to a 3:1 variable ratio schedule and progressively shifted to a final variable ratio of 10:1. As responding on the final schedule improved, deodorized air was passed through the response chamber. Once a stable response rate of 10:1 was observed, discrimination training was begun. Four channels were used, two for odorous solutions and two for blank, deodorized solutions. An odor was presented at an intense concentration (.3M) during which no reinforcement was available. If the subject stopped responding for from 8 to 10 sec., the odor was terminated and the reinforcement schedule was reinstated. However, if no suppression of bar pressing occurred, the odor and the withholding of water

continued for 2 min. Thereafter, odor was discontinued and reinforcement reinstated. On occasions, a blank rather than an odor was presented and reinforcement was continued. A trial (the presentation of an odor or a blank) occurred every 7 to 15 reinforcements for a total of 24 (12 odor and 12 blank) trials per session. Discrimination and, therefore, suppression was said to have occurred when not more than one bar press was made during an 8-sec. interval beginning 2 sec. after odor onset. The concentration was decreased by a factor of 10 when the animal discriminated on 10 of 12 odor trials for three consecutive sessions. When criterial discriminative performance was achieved at a solution concentration of 1 X 10^{-3}M, the procedure was altered. Previous experimentation had established that this concentration was approximately one and a half log steps above threshold for normal, intact rats.

Each of four odorant bottles was filled with a solution of different concentration (generally one-half log step difference between concentrations) and two bottles were used as blanks. A program was developed such that odor and blank stimuli were randomly presented with intertrial intervals averaging 30 sec. Each stimulus was presented for 15 sec., and the number of responses occurring during an interval beginning 2 sec. after stimulus onset and terminating 12 sec. after stimulus onset were recorded. The program was presented at least twice in each experimental session. An attempt was made to establish two points above and two points below threshold. Preliminary observations had indicated that a detection could be said to have occurred when the ratio of the number of responses during an odor over the mean number

of responses during the blanks for the session was .625 or less. Thus, a dichotomous classification was established. The concentration having a .50 probability of detection, so defined, was taken to be the threshold point. This point was considered to have been reliably identified when the subject's performance was stable over five consecutive sessions. Once this criterion was reached, the ADX subjects were administered corticosterone[3] and their limen reassessed. Since there was no existing data suggesting the adequate glucocorticoid injection for producing a rise in olfactory thresholds of ADX rats, various concentrations and preparations were tried which have been shown to return steroid blood levels to normal in operated rats (Hodges & Jones, 1964; Resko, 1970). Three milliliters of a 7% ethanol-saline preparation of the hormone at a concentration of .5 mg/ml were injected subcutaneously every 12 hr. (10:45 p.m. and 10:45 a.m.). This administration continued for 4 days. The dosage was then increased to 3 ml of a 1 mg/ml solution in a saline suspension, again injections being given every 12 hr. This maintenance was continued for 3 days, then terminated.

Following a 6-day, no-session-no-injection period, steroid therapy was reinstated. However, a new, fresh supply of corticosterone was purchased for this phase of the experiment. Here, subjects were injected subcutaneously every 12 hr. with 1 ml of a preparation of 6 mg/ml steroid in propylene glycol vehicle. Administration continued for 4 days, after which the dosage was increased. Then, for 5 days, 1 ml of 15 mg/ml steroid in propylene glycol

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[3]Steraloids, Inc., Pawling, New York.

was injected every 12 hr. Although steroid therapy was discontinued after the 5th day, 1 ml injections of propylene glycol vehicle were continued for 3 days.

Two of the normal, intact subjects were then selected for further research. One animal was bilaterally adrenalectomized and the other received a sham operation. Both subjects were put on saline maintenance in conjunction with the deprivation schedule. Approximately 18 hr. postoperatively and for 4 days thereafter, the olfactory thresholds of each subject were determined. On day 3, both animals were given two injections, 8 hr. apart, of .5 ml corticosterone (6 mg/ml) in propylene glycol. Twelve hours after the last injection, thresholds were again assessed.

Of critical importance throughout the above procedure was the utilization of deodorized water for cleaning glassware and preparing odorant solutions. Typically, such material is prepared by filtering glass distilled water through granule charcoal (Pfaffmann, 1971). It has been the author's experience, however, that this process fails to purify water to a degree commensurate with the potential purity of the olfactometer used. Therefore, an alternate procedure for obtaining pure water was adopted (Bircher, 1940). To 12 liters of metal-distilled water was added 1.5g of refluxed $KMnO_4$ and 4 ml of H_2SO_4. The solution was then distilled in a glass distilling apparatus. This step resulted in the removal of organic solutes which still remained in the single-distilled water. The middle one-third portion of the distillate was retained and boiled down to one-half of its volume in order to remove dissolved gases. Reserve quantities of water, taken from the middle one-third

distillation, not purified of gases, were maintained such that a desired quantity of pure water could be prepared daily simply by completing the final step.

RESULTS

The differences in detection thresholds for pyridine between intact and ADX groups were dramatic. Figure 3 shows the log median molar concentrations at threshold for each group. As can be seen, the ADX rats were able to detect pyridine at 1.0 log step lower intensity than the normal subjects. Also indicated in the figure is the range of thresholds observed for each group. For the ADX subjects the range was .7 log units, whereas for the normals a span of approximately 2 log units was recorded. The latter was related to the significant difference in median threshold between two subgroups of normals (see Figure 4). Because of the time required each day to train rats to suppress their ongoing responses in the presence of odor and due to the difficulty in maintaining purity within the olfactometer and animal chamber by daily disassembly and cleaning, the experimenter was restricted to training, assessing sensitivity, and, in general, completing all necessary research on only two animals at a time. Thus, thresholds were determined first for Normal Group I, followed by Group ADX, and finally Normal Group II. The data were transformed to $X = \sqrt{X}$ in order to effect a homogeneity in within-cell variances, and were then subjected to a two-way analysis of variance for repeated measures (Winer, 1962).

The difference in median threshold between Normal Group I and Normal Group II was not significant ($F = 10.99$; $df = 1,2$; $p > .05$), whereas Normal Group II displayed a much higher median threshold than the ADX group ($F = 33.0$; $df = 1,2$; $p < .05 > .01$). In all cases, within-subject

variability was not significant. As is indicated in Figure 4, there was some overlap in the range of thresholds between Normal Group II and the ADX group but not between the latter and Normal Group I.

The effects of steroid administration appeared unstable and inconclusive (Figures 5 and 6). Even though in some sessions subject #1465 gave threshold values above the highest observed in the pre-injection periods (44%), minimal values were lower than the bottom extension of the range (13%). Similar fluctuations by subject #1467 were seen except that here 73% of the readings were lower than the minimum of the pre-injection range, whereas only two thresholds (13%) surpassed the upper limit. There was no consistency observed in the direction of threshold shift in relation to steroid maintenance onset, duration or dosage. Although sensitivity increased in the three sessions during which vehicle only was administered, for both animals these values were equal to or greater than the minimums reached while under steroid conditions.

The consequences of adrenalectomy and later corticosteroid injections were more clearcut in Normal Group II. A sham operation had no alterative effects on the olfactory sensitivity of animal #1856 (Figure 7). Adrenalectomy of subject #1857 did produce a noticeable decrease in threshold. Further, corticosteroid administration failed to change the sensitivity of the sham operated subject, whereas such maintenance returned the threshold of the ADX animal to the value of the test day prior to surgery, although the limen did not shift to the median value of dates 2/1 through 2/5.

In order to determine whether adrenalectomy resulted in some general physiological debilitation which secondarily influenced olfactory performance,

the intertrial bar press response rate was calculated for both groups (Figure 8). There was no difference found between the ADX subjects and the Normal subjects ($t = 1.77$). Water ingestion was also monitored as an indicant of possible differential effects of the deprivation regimen on the two groups. The mean daily intake for 30 days, which included those periods during which thresholds were determined, are given in Table 1.

Finally, plasma corticosterone levels were determined for each animal using fluorometric analysis (Glick, Von Redlich, & Levine, 1964) and were found to substantiate the adrenohormonal state of the subjects expected from the operative procedure (Table 2).

DISCUSSION

The differences in odor sensitivity to pyridine between normal and ADX rats were significant. Although for adrenal insufficient humans the limen can shift by a factor of 100,000 times (Henkin, 1970), the 10-fold divergence observed in rats also leads to a proposition of some type of interaction between neural tissues and hormones besides that known for the control of elaboration and secretion of the substances themselves. The generality of the phenomenon has been fixed, at least for humans and rats. In the studies with human patients the experimental groups included individuals with hypophyseal pathology and, therefore, depressed ACTH levels, in order to determine whether the sensory alterations were mediated by ACTH or by glucocorticoids alone. The possibility existed that lowered thresholds in adrenal insufficiency were the result of increased ACTH secretions. Since detection thresholds for panhypopituitary subjects and adrenal insufficient patients did not differ, either during maintenance periods or when steroid therapy was terminated, an ACTH effect was ruled out. In the present study it cannot be unequivocally concluded that ACTH played no role in the differences seen. However, steroid maintenance was shown to have returned plasma corticosterone levels to normal range and most likely resulted in a reduction of the plasma ACTH concentrations to basal levels (Jacobs, 1971). Yet, as was seen in Figures 5 and 6, olfactory thresholds did not return to and remain at normal levels.

The differences found in olfactory thresholds between the two normal groups warrant consideration. Normal Group I consisted of two animals which had been trained in the early period of this study. At that time

acetic acid was used as the odorant and initially at rather high concentrations (.3 to .5M). It was also during this phase that necessary improvements in the olfactometer and its operation were introduced. Later, the odorant was changed to pyridine in order to achieve some similarity to the human studies, and threshold determinations were completed within 40 sessions. For Normal Group II, training was begun using pyridine, with an initial concentration of .01M. Here, a total of 146 sessions were given. At this stage of the research procedural competence had improved[4], and this taken together with the changes in training parameters, could account for the greater sensitivity recorded for Group II. Since the adrenalectomized subjects were trained just prior to Group II at a time when the suggested changes had already been introduced, these improvements would not account for the differences between ADXs and Normal IIs.

The response of the ADX animals to corticosterone maintenance was unstable. Although there was some indication that the threshold had temporarily increased beyond the maxima of the pre-injection variation, the limen also dropped to minimum values well below those observed before the injections. A comparison of the changes in response rate and fluctuations in thresholds during maintenance sessions revealed no relationships between these parameters. Neither can the alterations be necessarily explained in terms of inappropriate injection techniques or dosages. Fluorometric

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[4]Dr. David Phillips has indicated that it is not atypical in olfactory research for threshold values to appear to shift downward as experimenter experience and competence increases, reaching a stable point which thereafter seems to be unaffected by continued efforts.

analyses showed plasma corticosterone concentrations of ADX rats to be at normal levels following the steroid maintenance periods (see Table 2). The basis for these enhanced oscillations remains unclarified. It may be that glucocorticoids, and particularly corticosterone, are not involved in the observed olfactory alterations. Subject #9892 was functionally demedullate yet his median threshold was well above that of the ADX animals. Therefore, it is unlikely that the adrenal medullary hormones were involved in the threshold differences. The adrenal cortex, on the other hand, has been shown to elaborate over 40 compounds, including androgens, estrogens, progestogens, and corticoids (Short, 1960). The fact that in the present study corticosterone injections failed to return olfactory thresholds to normal levels may mean that one of the other adrenocortical hormones mediates the effect. It should be noted, however, that in humans the limen changes were related to glucocorticoid concentrations. Also, corticosterone in rats and hydrocortisone in humans are found to constitute the major proportions of the adrenal cortical secretions, that is, over 60% of the total steroid Ketol content. No other fraction present accounts for more than 6% (Soffer, Dorfman, & Garilove, 1961). Short concludes that it cannot be stated with certainty whether the observed secretion of androgens and estrogens is accidental, as a result of the measurement techniques, or purposeful. The adrenal must, therefore, be looked upon ... "as a 'leaky' gland, secreting many intermediates that may be of little consequence". There is considered to be an interplay between the gonads and the adrenals which is exemplified by the functional and anatomical changes in the reproductive system following

adrenalectomy (Soffer, et al., 1961). Thus, bilateral adrenalectomy in both female and male rats leads to atrophic changes in the gonads and presumably a decrease in the concentration of circulating sex hormones. Olfactory sensitivity following adrenalectomy may consequently be related to a depression in gonadal secretion, although such a conclusion is inconsistent with the observation that the olfactory thresholds for estrous female urine in normal and castrated male rats do not differ (Carr, Solberg, & Pfaffmann, 1962).

Since olfactory thresholds were not determined for the ADX group in infancy, that is before bilateral adrenalectomy, the question arose as to whether or not these two animals had inherent odor detection capabilities for pyridine greater than that of the normal subjects and unrelated to adrenal state. It was assumed that if that were the case then normal males adrenalectomized in adulthood following threshold assessment would have no change in sensitivity. The data did not support this assumption. The threshold of the adrenalectomized subject dropped to the range of those of the ADX group (#1465 and #1467) and remained there while the sham animal showed no shift. Further, steroid maintenance raised the ADX's threshold but had no effect on that of the intact animal. These results are consistent with the hypothesis of an adrenal mediation of sensory sensitivity.

The loss of adrenal hormones, even in the rat, leads eventually to a general debilitation manifested by a precipitous decrease in locomotor and grooming activity plus a rapid drop in body weight. However, a saline and dextrose maintenance program results in a return to normal of body weight and general activity. Normalization of performance of the bar pressing

task was observed for the ADX subjects during the entire period of their training. As is shown in Figure 8, these animals actually had a mean response rate during the intertrial periods slightly greater than the normals. The data supports the conclusion that ADX subjects had no muscular-capacitative deficit, at least for the task involved, which led to differences in response rate between these subjects and the Normals recorded during the test trials.

Ordinarily, a water deprivation regimen would be expected to lead to some degree of body dehydration and could, therefore, result in an alteration of epithelial nasal tissues. The effect would be magnified in adrenalectomized subjects due to their increased water loss and could in an unexplained way lead to differences in odor sensitivity. However, in the present study the watering schedule was gradually adjusted in order to increase body weight to 100% or greater of ad libitum values and consequently restore total water intake. Even though the subjects were given water only during a short period every 24 hours, the regimen resulted in a daily water intake for all animals which did not differ from that observed in intact and ADX rats on ad libitum food and water maintenance (de Wied, 1970).

It is also unlikely that the observed threshold shift was mediated by an alteration in olfactory mucus secretions because of the changes in glucocorticoid concentrations. Adrenalectomy will most likely lead to a small decrease in mucus secretions in many body areas and is probably related to the effects of the adrenal extirpation on systemic and cellular fluid and electrolyte balances (Bang & Bang, 1961), as well as to losses in available carbohydrates, the latter being an important fraction of mucus macromolecules

(Platt, 1966). Henkin, et al. (1966) have shown that restoring electrolyte balance will not return thresholds to normal in adrenal insufficient patients. Even if a dramatic mucosal change did occur as a result of adrenalectomy or adrenal pathology it would lead to an effect opposite to the one observed. The moist mucus surface is believed to be the medium in which olfactory molecules are dissolved (Bojsen-Moller, 1964) and maximal olfactory sensitivity is observed when the olfactory epithelium is red, swollen, and wet, provided that airways are not significantly obstructed (Schneider & Wolf, 1960).

It has been observed in humans that as glucocorticoid steroids are removed and an increase in sensory detection capabilities results, there is coincidentally a significant decrease in sensory integration for the modalities of taste and hearing (Henkin, 1970). Thus, recognition capabilities are markedly impaired but can be restored with appropriate steroid treatment. Henkin notes that glucocorticoids are a normal component of neural tissues and that the reciprocal changes in detection and perception are related to the manner in which specific steroids influence the metabolism of neural tissues. Adrenalectomy results in a significant decrease in the concentration of glucocorticoids in tissues of the central and peripheral nervous system. One effect of the resultant deficiency is to enhance the excitability of the nervous system such that normally subthreshold stimuli would produce neuronal depolarization. "This increase in neural excitability ... is hypothesized as the mechanism for the increase of sensory detection sensitivity observed in adrenocortical insufficiency."

It was noted above that Hoagland (1954) found a decrease, rather than an increase, in neural excitability related to adrenal deficiency.

Furthermore, Pfaffmann (1959) observed that the preference threshold for NaCl in the rat was lowered by adrenalectomy, yet electrophysiological investigations showed that there was no change in electric threshold as determined by single fiber recording. Either there were no excitability shifts or sensitivity changes were restricted to more central processes. Feldman & Dafny (1970) reported an increase in brain excitability in animals following the administration, rather than the depletion, of cortisol. Following the application of this hormone, sensory stimulation had a predominantly inhibitory effect on cell firing in the median eminance and anteromedian hypothalamus, while there was a facilitatory influence in the posterior hypothalamus.

Recent neuroanatomical, neurochemical, and electrophysiological evidence suggests an alternate explanation for the gross effects of adrenocortical hormones on sensory systems (Scott & Pfaffmann, 1967; Pfaff & Pfaffmann, 1969; Winans & Scalia, 1970; Pohorecky, Larin, & Wurtman, 1969; Powell, 1966; Girgis, 1969; Heimer, 1968; Raisman, 1966). An extensive centrifugal fiber system extending into the olfactory bulb has been traced in rats by Price (1969). These fibers arise from a discrete nucleus in the basal forebrain, the nucleus of the horizontal limb of the diagonal band. They pass from the nucleus to the posterior end of the lateral olfactory tract, turn rostrally and go into the olfactory bulb. At the posterior end of the bulb they fan out to enter the granule cell layer, from which they pass into the external plexiform layer and eventually ramify among the periglomerular cells (see also Kerr & Hagbarth, 1955). The nucleus in the diagonal band receives

afferent connections from the hypothalamus and midbrain. The termination of the centrifugal fibers is upon the spine-like projections (gemmules) of the peripheral granule cell processes. The gemmules have been shown to be the axonal components of a recripocal synapse found on mitral and tufted cell dendrites (Rall, Shepherd, Reese, & Brightman, 1966). Price (1968) suggests that these structures would provide a final common pathway for mitral cell inhibition and that the centrifugal fibers can influence directly this final path.

Within broadly defined areas of the septum and hippocampus of rats, McEwen, Weiss, and Schwartz (1969) found selective retention and a lowered rate of disappearance of corticosterone. The highest concentration in hippocampal tissue was observed in the most dorsal and anterior segment, nearest to the septum and fornix. Following adrenalectomy, the relative concentration of radioactive steroid in the septum remained constant for as long as 20 hours. This is consistent with the observation of a latency of several hours, once steroid therapy is terminated, for a shift in olfactory thresholds in humans (Henkin, et al., 1966). McEwen, et al. (1970) also reported steroid-specific retention by cell nuclei as opposed to cytoplasm. It has been proposed that the binding proteins regulate genetic activity in the cell nuclei by combining directly with regions of the genome or with other macromolecules which themselves are able to combine with the genome (Jensen, Suzuki, Numata, Smith, & DeSombre, 1969). The hormones are believed to alter the binding affinity of the protein for a region of the genome for the actual repressor molecule. The result of this action would be an increase in the activity of selective genes, leading to the production

of certain protein molecules which are necessary for cell function. Azmitia and McEwen (1969) have found the regulation of the level of tryptophan hydroxylase, a step in the biosynthesis of the neurohumoral agent serotonin, to be under the influence of corticosterone. The areas of the brain which were found to have the highest enzyme activity were, in descending order, the septum, hypothalamus, midbrain and thalamus. It was suggested that adrenocortical secretion controls *in vivo* activity of tryptophan hydroxylase (Azmitia, Costa, & Algeri, 1970).

The following mechanism is proposed as mediating the olfactory alterations observed in the present study: Corticosterone is preferentially bound to centrifugal neurons within the general area of the medial forebrain bundle; the result is an increased synthesis of neurohumoral fractions which in turn facilitates greater synaptic activity. In an adrenalectomized animal the inhibitory effects of these fibers on the olfactory bulbs are suppressed leading to an enhanced olfacto-centripetal action.

The suggested system can also account for alterations in olfactory activity observed during the menstrual cycle (Vierling & Rock, 1967) since it has been indicated that estrogens affect ACTH synthesis and/or release and consequently glucocorticoid levels (Richland, 1966).

SUMMARY AND CONCLUSIONS

Olfactory thresholds for pyridine were examined in normal and adrenalec-
tomized rats. The presentation of predetermined concentrations of odorant
was rigorously controlled through the utilization of an olfactometer, while
the recognition of the subjects' detection was realized by the establishment
of a discrimination-suppression response.

Prepuberally adrenalectomized (ADX) animals were found, in adulthood,
to have significantly greater olfactory sensitivity than normals. Although
there was also a difference in median threshold between two Normal subgroups,
this discrepancy was accounted for in terms of increased experimenter com-
petence as the study progressed, and the institution of changes in the
olfactometer which led to greater air purity.

The administration of corticosterone to the ADX group failed to reliably
shift their thresholds to Normal levels. One Normal subject was adrenalec-
tomized and a second was given a sham operation following threshold deter-
minations. The result was an increase in sensitivity for the former but no
change for the latter. Glucocorticoid administration had no effect on the
limen of the sham operated animal but served to shift that of the ADX toward
preoperative levels.

There were no differences seen in mean bar press rate between the ADX
and Normal groups. Although all subjects were kept on a 23.5-hour water
deprivation schedule and their body weights remained at approximately 100%
to 115% of ad libitum values, their daily water intake did not differ from

that observed in operated and intact rats maintained on an <u>ad libitum</u> food and water regimen.

It was concluded that the observed differences in olfactory sensitivity were unrelated to physical debilitation or olfactory-epithelium alterations resulting from adrenalectomy. Rather, the changes in odor detection were assumed to arise from the reduction of corticosterone concentrations in the nuclei of centrifugal cells which originate in the diagonal band and terminate in the olfactory bulbs.

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TABLE 1

Thirty-day mean daily fluid intake

Subjects	Preparation	Mean Intake CC/Day
1465	ADX	51.2
1467	ADX	59.4
1856	Normal	19.6
1857	Normal	18.1

TABLE 2

Mean plasma corticosterone concentration

Subjects	Date	Preparation	Mean μg/100ml
#448	7/17	Intact	11.0
#9892	7/17	ADX (7/14)	1.5
#9892	7/30	ADX	2.0
#1467	2/8	ADX (11/23)	3.5
#1467	2/28	ADX*	17.5
#1465	2/28	ADX*	30.5

*On steroid replacement

Figure 1. Response chamber and accessory apparatus.

    A. Response Chamber

    B. Diffusion Chamber

    C. Horizontal Bar

    D. Dipper Receiving Hole

    E. Speaker

    F. Air Inlet Tubes

    G. Enclosure Vent

    H. Exhaust Hose
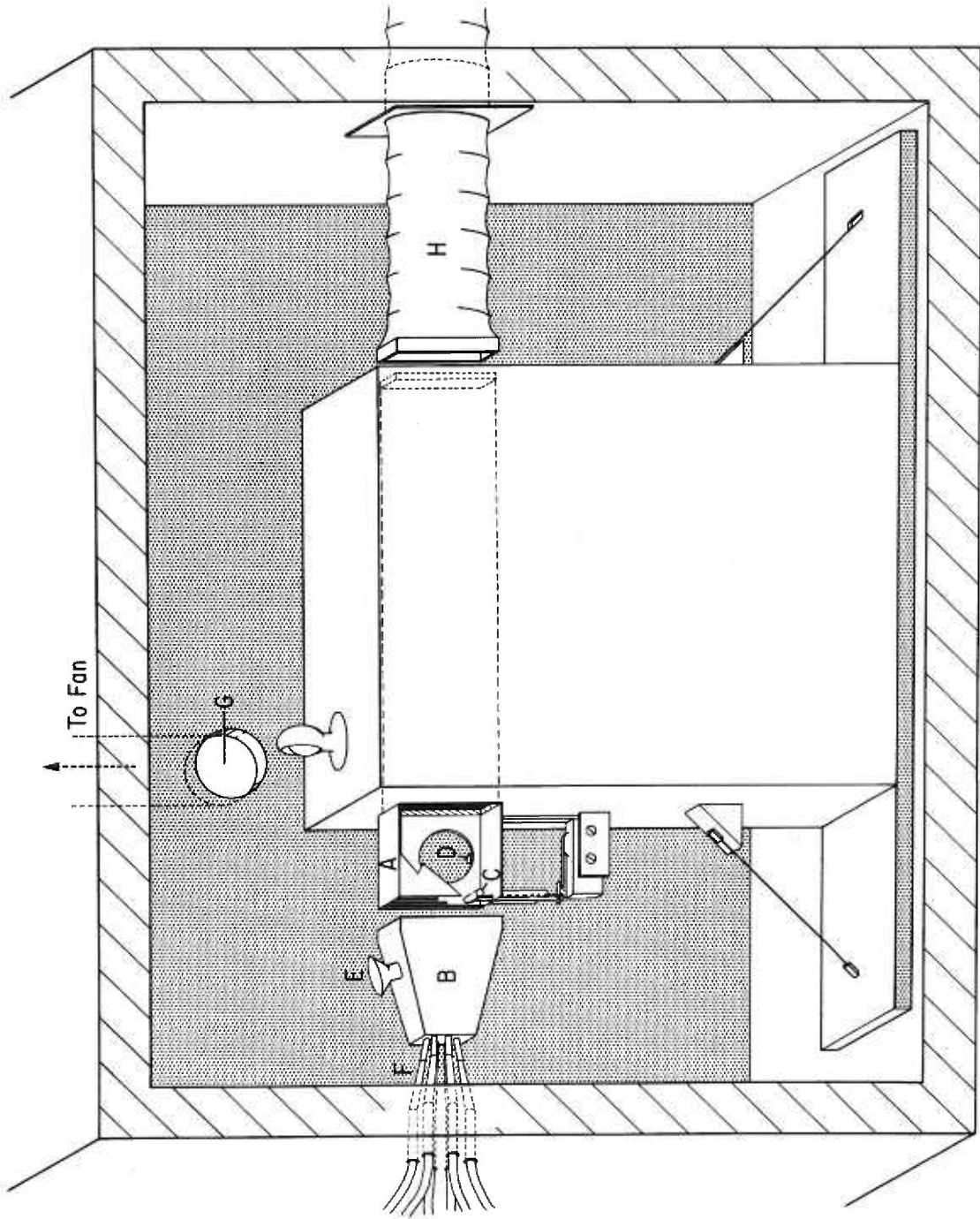
RESPONSE CHAMBER AND ACCESSORY APPARATUS

Fig. I

Figure 2. Schematic of the olfactometer.

A. Air Supply

B. National Cylinder Gas Regulators

C. 20-Liter Carboys

D. Purafil Filter

E. Calcium Chloride Dryers

F. Charcoal-Silica Gel Filters

G. 8-ft. Glass Coils

H. Flowmeters

I. Manifolds

J. Microregulating Valves

K. Gas Diffusion, Odorant Bottles

L. 3-Way Glass-Teflon Stopcocks

M. Glass Ts

SCHEMATIC OF THE OLFACTOMETER
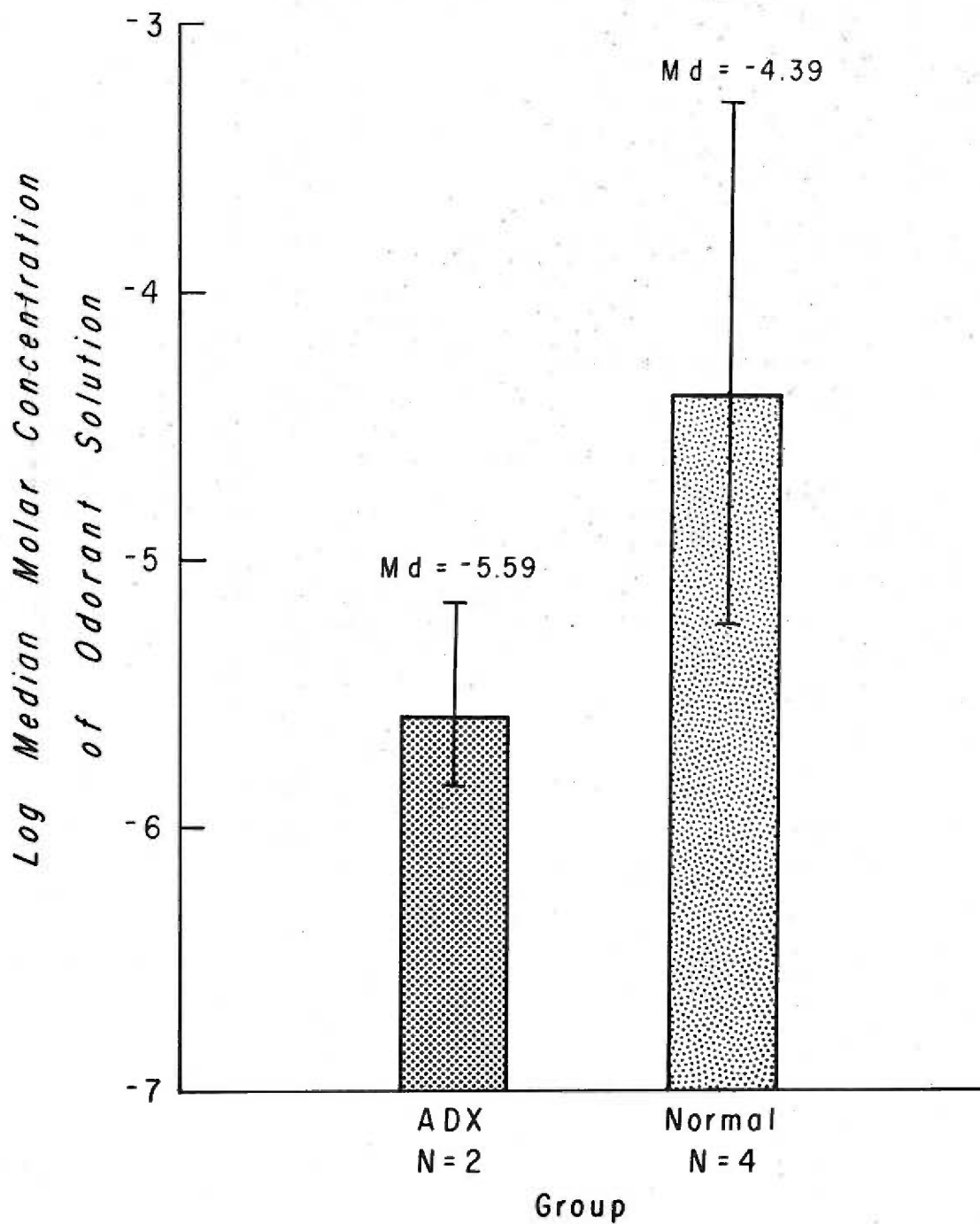
Water Bath
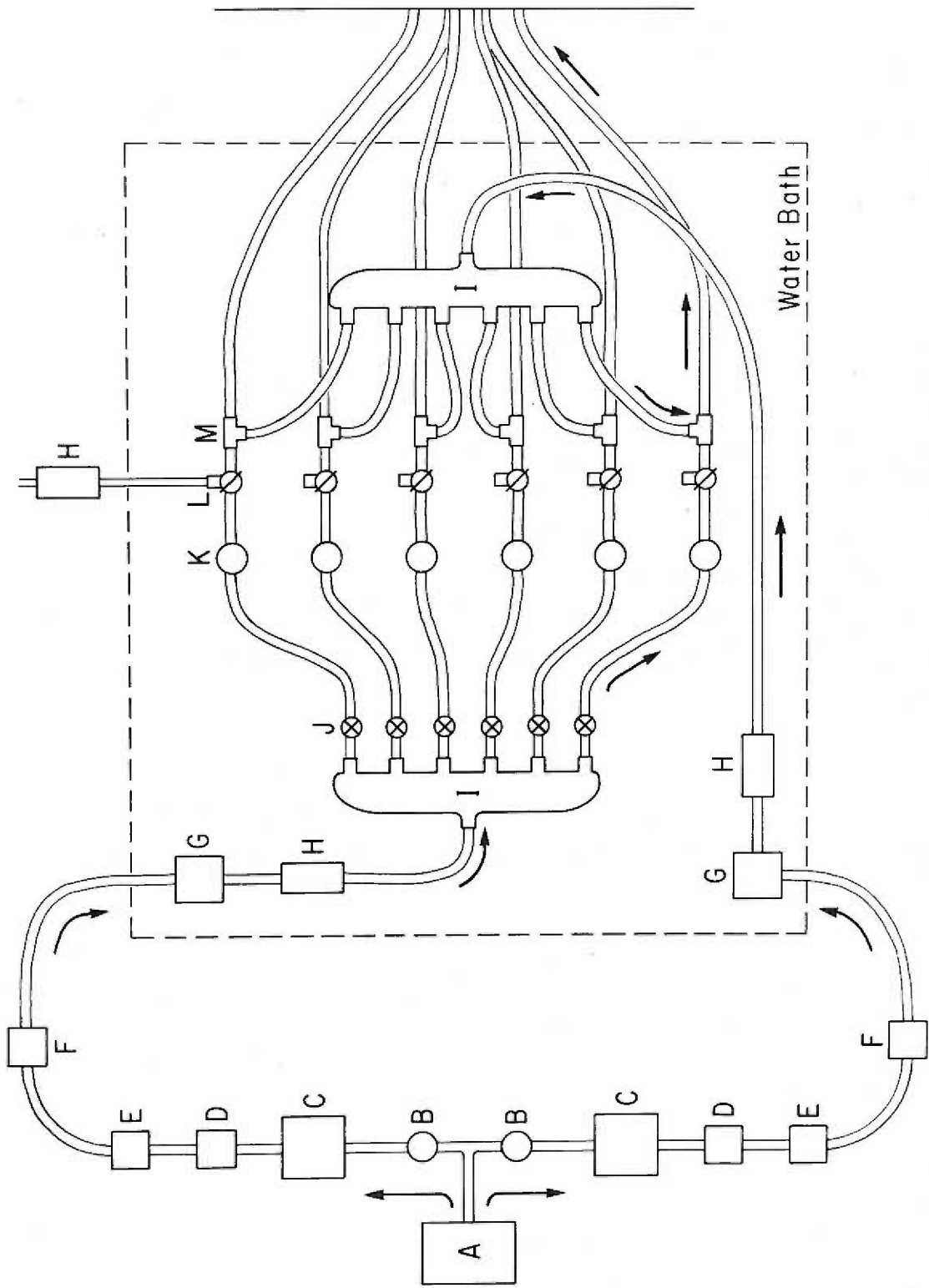
Fig. 2

OLFACTORY THRESHOLDS FOR PYRIDINE

Fig. 3

OLFACTORY THRESHOLDS DURING STEROID TREATMENT FOR SUBJECT #1465
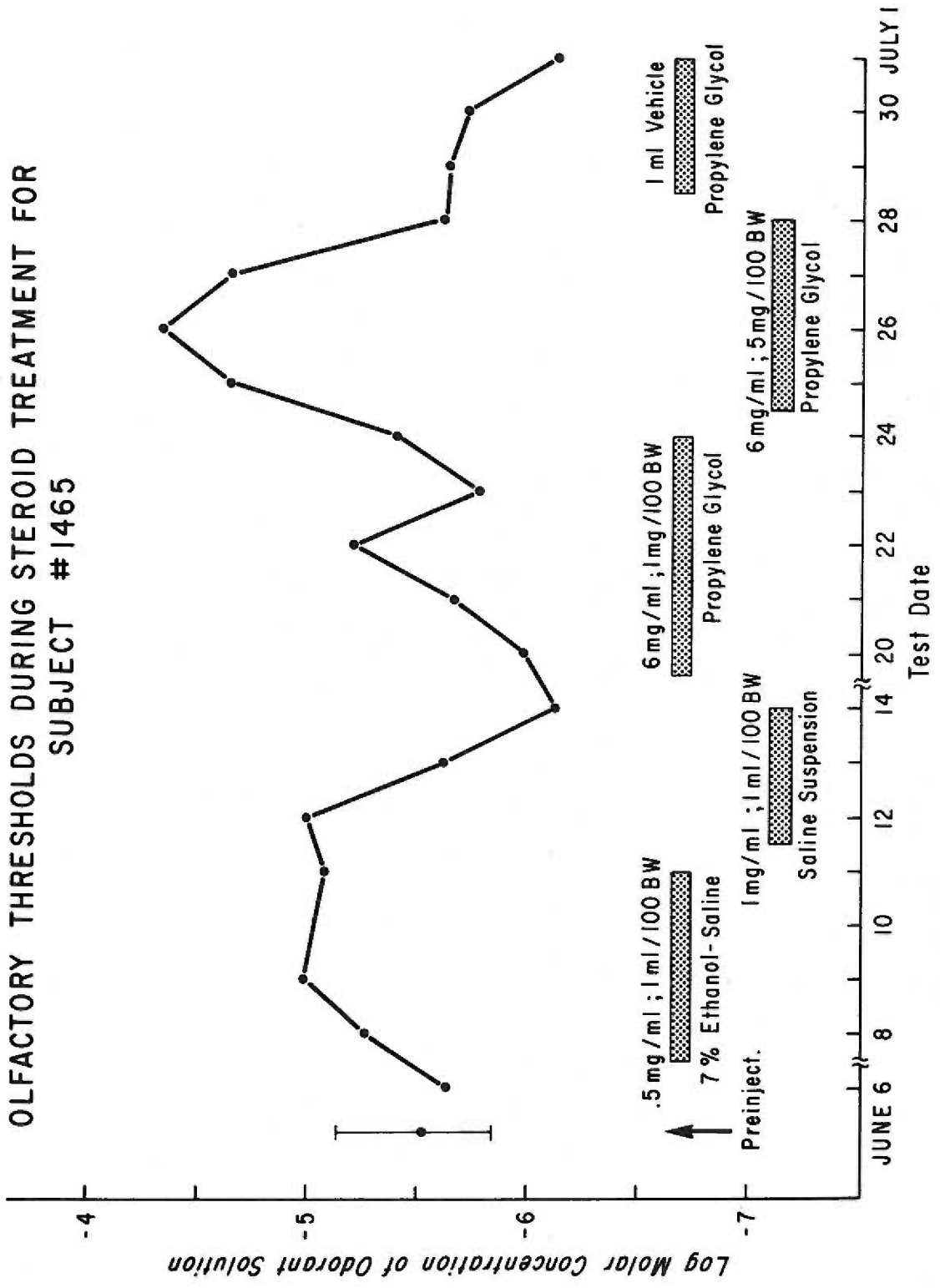
Fig. 5

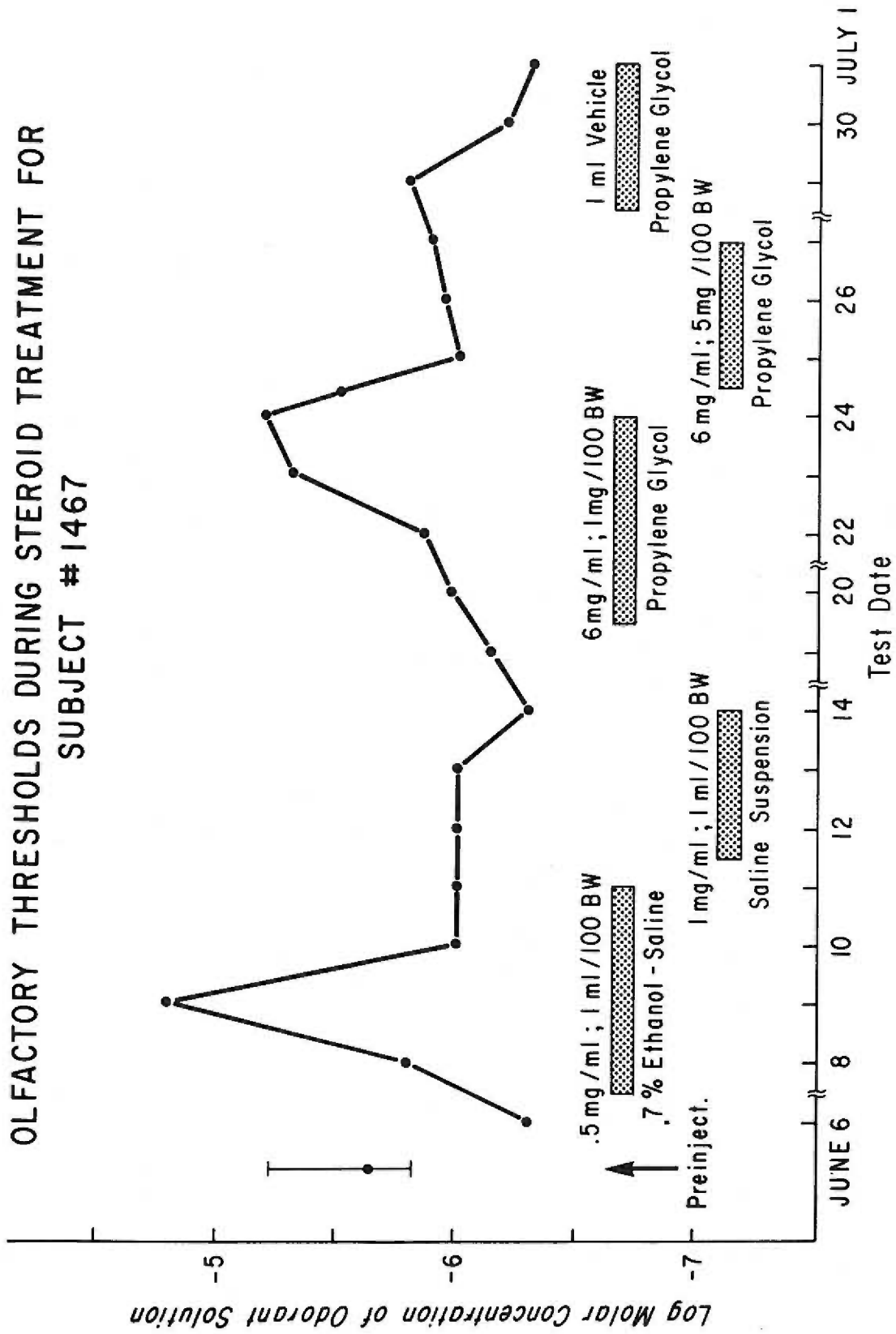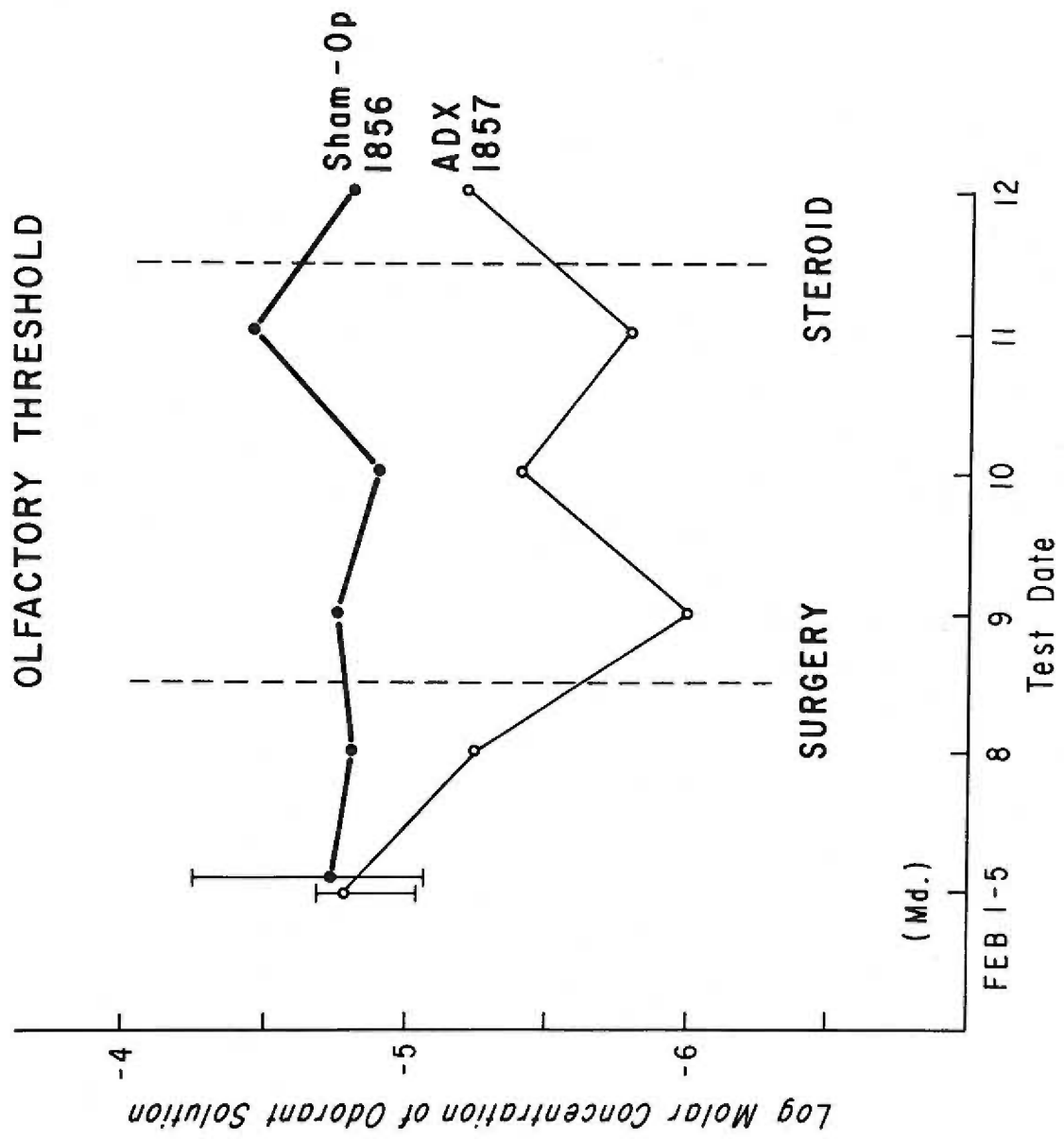OLFACTORY THRESHOLDS DURING STEROID TREATMENT FOR SUBJECT #1467

Fig. 6

OLFACTORY THRESHOLD

Fig. 7

MEAN BAR-PRESS RATE
(30-Session)

Mean Barpresses per Minute for Intertrial Periods

100 80 60 40 20

ADX   Normal

Group

Fig. 8