Preliminary experiments performed on mammalian liver cells (guinea-pig) showed similar results, that is, ouabain (6.8×10^{-7} *M*) reduced cell communication by 60% in about 10 min.

Uncoupling of heart cells produced by intracellular sodium injection was achieved more rapidly in presence of ouabain. As is illustrated in Figure 2A (average from 3 experiments) the injection of sodium into a Purkinje cell abolished the electrical coupling in 500 sec. Experiments performed on the same fibres exposed to ouabain (6.8×10^{-7} *M*) showed that the intracellular sodium injection caused uncoupling in 230 sec (see Figure 2B – average from 3 experiments). These results are probably explained by the build up of a larger intracellular sodium concentration in a shorter period of time since the extrusion of sodium was reduced or abolished by ouabain. In both situations the input resistance of the injected cell was increased as is shown in Figure 2, C and D.

Stimulation of the heart fibres at a high rate (3 c/s) that is known to raise the intracellular sodium concentration also lead to accentuated decrease of cell communication in Purkinje fibres exposed to ouabain. On these experiments, the hyperpolarization usually elicited by stimulation at a high rate in normal fibres[15] was negligible or absent, and in some experiments a depolarization was found, what probably contributed to the reduction of cell communication by increasing the inward movement of calcium.

In summary, the results presented above indicate that the sodium pump plays an important role on the regulation of cell communication in heart muscle. These observations highly suggest that the block of impulse conduction caused by cardiac glycosides in cardiac tissues, can be due, at least in part, to an increase in junctional resistance. The mechanism by which ouabain impairs cell communication in Purkinje fibres is probably related to the increase of the intracellular calcium content that follows the increment of the internal sodium concentration. A similar effect of ouabain in ventricular muscle has recently been reported by WEINGART[16]. The fall of intercellular communication found in liver cells exposed to the glycoside seems to indicate that the role of sodium extrusion on maintaining a high conductance pathway between cells is not limited to cardiac muscle.

An important implication of these results is that the intercellular movement of ions and molecules[17] can be largely reduced or abolished by suppression of the sodium pump. The physiological and pathological meaning of these observations are obvious and requires further investigation.

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Behavior and Endocrine Effects of 3,4,5-Trimethoxyamphetamine in Male Mice

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Summary. Effects of single doses of 50 and 100 mg/kg of TMA given i.p. were noted in male albino mice after 40 min and $2^1/_2$ h. Locomotor activity was significantly altered and biochemical tests indicated stimulatory effects on adrenocortical and adrenomedullary functions due to TMA.

In previous studies SACKLER et al. have investigated behavioral and endocrine effects of LSD-25[1,2], and mescaline[3,4] in laboratory animals in expectation that pertinent data could be obtained concerning possible biochemical basis in the etiology and pathophysiology of schizophrenias. PERETZ et al.[5] first reported that a derivative of amphetamine namely 3,4,5-trimethoxyamphetamine (TMA) was also hallucinogenic in man producing effects similar to its close chemical, psychomimetic relative mescaline. Chronic intake of amphetamine has similarly been known to cause paranoid psychoses in certain addicts resembling schizophrenia[6]. SHULGIN[7] testing a number of TMA derivatives among them the present 3,4,5-analogue reported the drug was twice as active as mescaline. The present investigation therefore sought to determine acute behavioral, biochemical and adrenal (adrenocortical and adrenomedullary) influences of 3,4,5-trimethoxyamphetamine in male albino mice to observe a possible common pattern and relationships of hallucinogenic substances.

The compound 3,4,5-trimethoxyamphetamine HCl (TMA) was synthesized by the method of HEY[8]. To observe acute effects of TMA on behavior and endocrine activity, male albino mice (CFW) averaging 25 g were matched by body weights into appropriate test and control groups after prior acclimatization for 1 week in cages containing 4 mice per cage. Test animals were injected i.p. with TMA solutions at dose levels of 50 mg/kg (Group A) and 100 mg/kg (Group B). Control mice received equivalent injections of saline.

Effects on locomotor activity were evaluated in open-field enclosures[4] for 0–40 min at 5 min intervals in aliquot groups of mice and in additional groups from 40 to 150 min after administration of the single injections of TMA and/or saline solutions. Aliquot groups of test and control mice were likewise sacrificed by rapid decapitation 40 min and $2^1/_2$ h after TMA administration. Heparinized blood specimens were collected and assayed for plasma glucose[9] and corticosterone[10] titers. The adrenals were excised

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Table I. Effects of 3,4,5-TMA on locomotor activity of male mice during 40 min

Group	Dose (mg/kg)	*n*	Number of lines crossed during 5 min periods								Total
			0–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	0–40
Group A ± SE	50	14	108.6 ± 11.8	65.1 ± 8.7	34.4 ± 5.5	31.8 ± 6.1	40.2 ± 7.8	43.9 ± 10.0	48.8 ± 8.9	62.2 ± 14.1	435.1 ± 54.3
Group B ± SE	100	14	91.1 ± 8.3	48.0 ± 10.9	30.0 ± 4.1	32.7 ± 4.3	41.5 ± 4.5	53.2 ± 6.0	69.5 ± 7.0	80.7 ± 8.2	446.8 ± 39.0
Group C ± SE	saline	14	82.0 ± 7.0	61.1 ± 6.4	41.6 ± 7.8	47.4 ± 6.0	45.4 ± 8.2	45.6 ± 8.7	35.4 ± 4.2	38.4 ± 5.9	396.7 ± 36.1
% difference A vs C			+ 32.3	+ 6.5	− 17.3	− 32.9	− 10.9	− 3.7	+ 37.9	+ 62.0	+ 9.7
P-value			0.07	0.71	0.47	0.08	0.67	0.90	0.19	0.14	0.56
% Difference B vs C			+ 11.0	− 21.3	− 27.9	− 31.0	− 8.0	+ 16.7	+ 96.3	+ 110.2	+ 12.6
P-value			0.42	0.31	0.22	0.06	0.70	0.48	< 0.001	< 0.001	0.36
% Difference A vs B			− 16.1	− 26.1	− 12.8	+ 2.8	+ 3.2	+ 21.2	+ 42.4	+ 29.7	+ 2.7
P-value			0.24	0.24	0.53	> 0.90	0.89	0.43	0.08	0.27	0.86

Table II. Effects of 3,4,5-TMA on locomotor activity of male mice during 40–150 min

Group	Dose (mg/kg)	*n*	Number of lines crossed during 5 min periods							
			46–50	56–60	80–85	86–90	110–115	116–120	140–145	146–150
Group A ± SE	50	14	78.0 ± 12.0	75.2 ± 11.0	74.2 ± 10.4	71.5 ± 10.4	65.1 ± 14.7	36.5 ± 10.8	33.3 ± 11.6	25.6 ± 9.0
Group B ± SE	100	14	122.9 ± 16.1	117.2 ± 16.0	133.4 ± 12.8	116.1 ± 15.2	112.1 ± 20.6	112.1 ± 21.2	75.1 ± 12.3	63.1 ± 9.7
Group C ± SE	saline	14	43.1 ± 8.0	26.1 ± 6.6	5.6 ± 2.6	5.1 ± 4.2	3.7 ± 2.3	1.6 ± 1.5	8.1 ± 3.7	8.4 ± 4.5
P-value A vs C			0.02	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01	0.05	0.10
P-value B vs C			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P-value A vs B			0.04	0.04	< 0.01	0.02	0.08	< 0.01	0.02	< 0.01

Table III. Effects of 3,4,5-TMA on plasma corticosterone and glucose and adrenal epinephrine levels of male mice

Group	Dose (mg/kg)	40 minutes						$2^1/_2$ h					
		n	Plasma corticosterone (μg/100 ml)	*n*	Plasma glucose (mg/100 ml)	*n*	Adrenal epinephrine (μg/100 mg)	*n*	Plasma corticosterone (μg/100 ml)	*n*	Plasma glucose (μg/100 ml)	*n*	Adrenal epinephrine (μg/100 mg)
Group A ± SE	50	12	56.91 ± 2.69	8	216.62 ± 12.45	13	135.34 ± 13.05	14	32.15 ± 4.32	11	147.35 ± 8.53	14	173.49 ± 12.51
Group B ± SE	100	14	60.01 ± 3.88	11	200.75 ± 9.36	14	125.42 ± 7.90	13	39.90 ± 2.78	10	158.98 ± 7.34	14	165.52 ± 11.11
Group C ± SE	saline	14	41.94 ± 9.33	12	169.12 ± 8.52	14	148.79 ± 6.82	14	23.61 ± 2.43	13	133.26 ± 6.19	14	182.94 ± 8.93
% Difference A vs C			+ 35.7		+ 28.1		− 9.0		+ 36.2		+ 10.6		− 5.2
P-value			< 0.001		< 0.001		0.36		0.10		0.19		> 0.90
% Difference B vs C			+ 43.1		+ 18.7		− 15.7		+ 69.0		+ 19.3		− 9.5
P-value			< 0.001		0.02		0.04		< 0.001		0.01		0.78
% Difference A vs B			+ 5.4		− 7.3		− 7.3		+ 24.1		+ 7.9		− 4.6
P-value			0.53		0.31		0.52		0.16		0.32		0.88

and similarly assayed for measurements of adrenal epinephrine levels[11].

Table I presents the locomotor activity in terms of number of lines crossed by the test and control mice at 5 min intervals during the 0–40 min period. It is apparent that during the first 5 min, TMA initially caused a transitory stimulation of locomotor activity followed by decreased activity which tended to be most marked during the 16–20 min period. None of the alterations were statistically significant by Student's *t*-test procedures[12], although *p*-values were low with both doses during the 16–20 min period (Group A – 0.08; Group B – 0.06).

Thereafter, revealing further relationships to dosage, inhibitory effects on locomotor activity declined and at the 31–35 and 36–40 min periods significant increases were observed in the locomotor activities of the 100 mg/kg treated mice. Corollary increases induced by the 50 mg/kg dose at those periods were not statistically significant. It should be noted that evaluation of total locomotor activity during the 0–40 min period would not have indicated the various changes in the locomotor activity patterns of the TMA-treated mice since the summed effects in both test groups revealed increases in locomotor activity which were not statistically significant for the total 40 min period.

In contrast, continued evaluation of the effects of the hallucinogenic agent TMA on locomotor activity of different populations of test vs. control mice after 40 min to $2^1/_2$ h intervals, demonstrated consistent and in general significant increases in the subsequent locomotor activity of both test groups with the 100 mg/kg dose causing significantly greater increases than even the 50 mg/kg dose. Table II presents some representative samplings of locomotor activity patterns observed at 5 min periods during the 40 min to $2^1/_2$ h post-injection and observation periods. It should be noted that at 80 min and thereafter, the locomotor activity of the control group had declined considerably due to the generalized lesser activity in the control group. Thus, 11 of the control mice during the 80–85 min period were relatively inactive, assuming quiet and resting postures as compared to 7 in Group A (50 mg/kg) and 1 in Group B (100 mg/kg). These findings would indicate that TMA significantly stimulated locomotor activity during the 40–150 min observation period and appeared to attain peak levels at the 80–85 min interval suggesting an amphetamine-like effect on locomotor activity.

Table III presents plasma corticosterone and glucose as well as adrenal epinephrine levels of the test and control groups sacrificed after 40 min and $2^1/_2$ h. It is evident that at 40 min both doses caused significant increases in plasma corticosterone and glucose values. Although decreases were observed in adrenal catecholamine titers of both test groups, a significant reduction in adrenal epinephrine was only caused by the higher dose.

After $2^1/_2$ h, persistent increases were still evident in plasma corticosterone and glucose test group levels but only the 100 mg/kg dose showed a statistically significant increase. In contrast, none of the decreases in adrenal epinephrine induced by both doses at $2^1/_2$ h were statistically significant. Indicative of the greater activity of the higher dose, the percent changes caused by the 100 mg/kg dose in glucose, corticosterone and epinephrine were approximately 2 times greater than the percent changes evoked by 50 mg/kg, although the differences between Groups A and B were not significant.

It is apparent that acute administration of 3,4,5-trimethoxyamphetamine in the male mice produced stimulatory effects on adrenocortical and adrenomedullary activities causing respective release of corticosterone and epinephrine. The adrenocortical effects resemble actions of non-specific stress agents similarly noted in our prior LSD-25[1,2] and mescaline[3,4] investigations. The significant increase in plasma glucose is likewise in accord with non-specific stress reactions and reports that adrenal catecholamine release stimulates hyperglycemia[13]. In conclusion, 3,4,5-TMA evoked significant alterations in locomotor activity and stimulated adrenal activity.

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An Amnesic Effect of Benzodiazepines in Rats?

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Summary. In rats, benzodiazepines seem to induce some amnesia as reported in man. This effect is apparent during the learning of both noxious events and extinction only if the rats are under the drug influence during the conditioning session.

It has recently been reported that diazepam[1–3], a benzodiazepine derivative, has an amnesic effect in man. Although such an activity has never been demonstrated in animal studies, it could contribute to the effect of benzodiazepines in some experimental procedures. In animals given chlordiazepoxide prior to learning a conditioned emotional response (CER), no behavioural inhibition was seen when a further conditioned stimulus appeared[4,5]. This effect does not seem to result only from a state-dependent learning[4,5], but it could be explained by the attenuation of the conditioning properties of the shock, since chlordiazepoxide has been shown to modify pain sensitivity or the emotional state elicited by pain[6].

In a similar protocol of CER we have examined the effect of 3 benzodiazepines (lorazepam, diazepam and chlordiazepoxide) and 1 neuroleptic (chlorpromazine) on behavioural inhibition by administering these agents before the learning phase (protocol A). In order to test if

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