Gm. (0.1 mole) of 3,4-dimethoxyphenethyl amine, and 150 ml. of benzene was refluxed on a steam bath for 6 hr. The benzene was evaporated and thereafter the dark brown oily residue was heated on a steam bath for 6 hr. The residual material solidified on cooling.

2-Benzylaminomethyl-1-indanone (XVI)—To a mixture of 14 ml. of concentrated hydrochloric acid and 14 Gm. (0.13 mole) of benzylamine were added 18.5 Gm. (0.14 mole) of 1-indanone and 12 ml. (4.1 Gm., 0.14 mole) of 37% formaldehyde solution. The resulting mixture was heated on a steam bath for 40 min. and thereafter cooled and filtered. The residual material was washed with two 50-ml. portions of methanol to give 20 Gm. (53%) of white solid, m.p. 190–193° dec.

2 - Dimethylaminomethyl - 4 - (1 - indanyl)-phenol (XVII)—A solution of 21 Gm. (0.1 mole) of 4-(1-indanyl)phenol in 100 ml. of isopropanol was treated with 18 ml. of 25% aqueous dimethylamine solution and 10.5 ml. of a 37% formaldehyde solution and thereafter refluxed on a steam bath for 1.5 hr. The solvent was distilled *in vacuo*. The residue was treated with dilute hydrochloric acid and extracted with ether. The acid solution was neutralized with ammonium hydroxide and extracted with ether. The latter ether extract was dried over anhydrous magnesium sulfate and then evaporated to dryness. The residual material (10 Gm., 40%), which melted at 51–56°, was recrystallized.

2-Hydroxy-3-methoxybenzylmethylamine (XVIII)—The procedure for the preparation of X was followed using 31 Gm. (0.7 mole) of 2-hydroxy-3-methoxybenzalmethylamine. Reduction was complete after 18 hr.

N - Benzyl - N - (2 - indanylmethyl)methylamine (XIX)—A mixture of 10.6 Gm. (0.05 mole) of 2-bromomethylindan and 25 Gm. of *N*-benzylmethylamine was refluxed for 6 hr. Thereafter the mixture was cooled and extracted with dilute hydrochloric acid. The acid solution was neutralized and extracted with ether. The ether extract was dried over anhydrous magnesium sulfate and then distilled to give product.

A hydrochloride (XX) was prepared in the usual manner and recrystallized.

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Placebo Effect of Saline on Locomotor Activity in Several Strains of Mice

By W. M. DAVIS, W. T. KING, and M. BABBINI

The effect of intraperitoneal injection of 0.9 per cent saline solution upon locomotor activity of six strains of mice was observed in actometer cages. Whether tested singly or in groups of four, a depressant effect of saline upon activity was seen. The strains differed significantly in activity levels, but not in degree of effect of saline on activity. Group testing had differing degrees of effect on activity counts compared to single-mouse tests in the several strains.

A REDUCTION in the locomotor activity of mice which received an injection of physiological saline in comparison to the activity of uninjected mice has been reported by Schnitzer and Ross (1). They found that this effect was dependent upon more than the act of handling the mouse or handling plus intraperitoneal insertion of a hypodermic needle. Meier (2) confirmed their findings with 0.9% saline group compared to a sham-injected group even while using only one-half the volume (0.005 ml./Gm.) administered by the former workers. Schnitzer and Ross (3) later failed to observe an inhibitory effect of saline when they used younger mice than those of their first report; however, testing at a higher room temperature may have been responsible for the difference. Meier's confirmation was by means of younger mice than were used in either of the other reports.

Meier *et al.* again reported inhibition of locomotion by saline injection incidental to other observations involving four inbred strains of mice (4). They stated that the ranking of post-saline activities of the strains was significantly different from the ranking of activities in a previous noninjection test, but without presenting fully the data to describe this finding. Further evidence regarding the genetic factor as a possible variable in the "placebo effect" of saline in-

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TABLE I—EFFECTS OF SALINE INJECTION ON LOCOMOTOR ACTIVITY OF SIX STRAINS OF MICE TESTED SINGLY OR IN GROUPS OF FOUR

Mean 30-min. Activity Count[a] (Log-Transformed)					
Single Mice			Grouped Mice		
Strain	No Inject.	Saline	Strain	No Inject.	Saline
NLW	2.990	2.810	NLW	3.522	3.409
C57BL/6	2.948	2.904	C57BL/6	3.452	3.360
DBA/2	2.934	2.855	C3H/An	3.381	3.331
BALB/c	2.913	2.905	CBA	3.335	3.353
C3H/An	2.847	2.796	DBA/2	3.324	3.235
CBA	2.778	2.714	BALB/c	3.322	3.300

[a] $N = 12$ in all cases.

RESULTS

Locomotor activity values under the several treatment conditions are shown in Table I. Results of the analysis of variance performed on the log-transformed data[4] are shown in Table II. The analysis revealed significant main effects of both saline injection ($p < 0.005$) and of strains ($p < 0.005$), and quite expectedly, of grouping ($p < 0.005$).

In view of the nonsignificant interaction of injection and strains, further analysis of the injection effects within strains was unwarranted. Further details of the between-strains differences contributing to the significant strains effect were determined by means of Duncan's multiple range test (8). This test was performed separately on the data for singly-tested and group-tested mice because of the significant interaction of strains and groupings, but after combining injected and noninjected groups in view of the nonsignificant interaction of injection with strains. Results of these multiple comparisons between strains are given in Table III. The significant interaction between grouping and strains, along with obvious difference in ordering of the strains under single and group tested conditions (Table III) suggested also the application of Duncan's test to the differences for each strain between values of single and grouped mice. The results of this test (Table IV) indicate that in addition to the differences in motility between strains as shown in Table III there is a significant difference in the effect of grouping on motility in different strains. The obvious and expected increase in counts when four mice are tested rather than one was in fact significantly more pronounced in the CBA, NLW, and C3H/An strains than in the DBA/2 and BALB/c. This suggests that the increase of counts with grouping is, for some strains at least, not merely a physical phenomenon as a consequence of the increased number of mice in the actometer, but also a behavioral phenomenon involving social factors.

TABLE II—ANALYSIS OF VARIANCE ON LOG-TRANSFORMED ACTIVITY DATA

Term	Degrees of Freedom	Sum of Sq.	Mean Sq.	F	p
Treatments	(23)	19.381	0.842	30.07	<.005
Injection	1	0.280	0.280	10.00	<.005
Grouping	1	17.763	17.763	634.39	<.005
Strains	5	0.695	0.139	4.96	<.005
Injection × grouping	1	0.001	0.001	<1	N.S.
Injection × strains	5	0.115	0.023	<1	N.S.
Grouping × strains	5	0.493	0.098	3.50	<.01
Injection × grouping × strains	5	0.034	0.006	<1	N.S.
Error	264	7.482	0.028	...	...
Total	287	26.863	0.093	...	...

jection on locomotor activity of mice seemed desirable. Since spontaneous locomotor activity is well known as a behavioral phenotype which is influenced by genetic differences between strains of laboratory mice (5, 6), it is of particular interest whether the effect of saline injection would occur independently of genotype. As all the previous reports of this phenomenon were based on observations of individual mice, it also seemed desirable to test for the effect in the case of small groups as are often used in studying locomotor effects of drugs. In summary, locomotor activity of groups of four mice and of single mice from six strains under noninjection and saline-injection conditions have been compared.

EXPERIMENTAL

Observations of locomotor activity were made in three commercially manufactured[1] photocell activity devices (7). All actometer units were exposed to each level of all the experimental variables. The number of counts, each representing the interruption of one of the six light beams distributed symmetrically around the ring-shaped track, was recorded by digital counters.

Inbred male mice of the C57BL/6, DBA/2, C3H/An, CBA, and BALB/c strains[2] and random-bred male mice of a Swiss-Webster albino strain (NLW)[3] were received at about 30 days of age and were between 8 and 12 weeks old when 60 from each strain were used in these experiments. A 0.9% saline solution in distilled water was administered intraperitoneally in a volume of 0.01 ml./Gm. Locomotor activity recording was begun immediately after injection and continued for 30 min. The handling of noninjected mice was approximately the same as for the saline group, but sham-injection (*i.e.*, needle penetration) was not performed as Schnitzer and Ross (1) had found needle insertion had little or nothing to do with reduction of activity.

DISCUSSION

The earlier reports (1, 2, 4) of the activity-inhibiting effect of intraperitoneal saline treatment in mice are further confirmed and extended by these positive results. The question of age as a factor in the effect which was suggested by one negative report (3) is contradicted since the mice used in this case were also considerably younger than those of the initial report (1). An additional attribute of this

[1] Woodard Research Corp., Herndon, Va.
[2] Cumberland View Farms, Clinton, Tenn.
[3] National Laboratory Animal Co., Creve Couer, Mo.

[4] Log-transformation was performed in order to eliminate the proportionality between the means and variances of single mice *versus* groups of mice that occurred in the untransformed data.

TABLE III—RANKING AND COMPARISON OF MEAN LOCOMOTOR ACTIVITY OF SIX STRAINS OF MICE COMBINED ACROSS NONINJECTION AND SALINE TREATMENTS

	Mice Tested Singly[a]					
Strain	CBA	C3H/An	DBA/2	NLW	BALB/c	C57BL/6
Activity	2.733	2.821	2.894	2.900	2.908	2.926

	Mice Tested in Groups[a]					
Strain	DBA/2	BALB/c	CBA	C3H/An	C57BL/6	NLW
Activity	3.820	3.311	3.344	3.356	3.406	3.465

[a] Values not sharing underline differ significantly ($p < 0.05$).

TABLE IV—COMPARATIVE EFFECTS OF GROUPING ON ACTIVITY IN SIX STRAINS MEASURED AS MEAN DIFFERENCES FROM SINGLY TESTED MICE[a]

Strain	DBA/2	BALB/c	C57BL/6	C3H/An	NLW	CBA
Difference	0.386	0.403	0.480	0.535	0.565	0.611

[a] Values not sharing underline differ significantly ($p < 0.05$).

phenomenon indicated by these data is its occurrence in mice tested in groups, as well as those tested singly. Although the present observations include four of the same inbred strains utilized in the study by Meier *et al.* (4), the data do not confirm their implication that the effect of saline treatment varies between strains. While there was a superficial suggestion in the untransformed data that the degree of response to saline might differ between strains, the nonsignificant injection x strain interaction term failed to support such an inference. Any such trend surely was not consistent enough to be detected by the analysis.

The pharmacological basis for the phenomenon of saline inhibition of activity remains quite unknown. The authors have termed it a placebo effect following the definition of Wolf (9), "... any effect attributable to a pill, potion or procedure, but not to its pharmacodynamic or specific properties." It surely cannot have the same explanation as that given for a placebo effect of saline injection upon locomotor activity of rats (10), *i.e.*, simple Pavlovian conditioning of the depressant effect of a previously administered pharmacologically active agent, scopolamine.

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Cerebral Drug Metabolism Investigated by Isolated Perfused Brain *In Situ*

By G. BENZI, F. BERTÉ, A. CREMA, and G. M. FRIGO

The *in situ* isolated brain, supplied by an extracorporeal pump-oxygenator system, shows a drug metabolizing activity. The tested substances (aminopyrine and oxazepam) show a disappearance from the extracorporeal blood of dog and monkey related to the metabolic transformations (demethylation, acetylation, or glucurono-conjugation) and to the fixation at the cerebral tissues.

IN A PREVIOUS PAPER (1) the authors described a method to investigate *in situ* the metabolizing activity of the liver connected normally with the body, except for blood circulation, which was supplied by a pump-oxygenator system. In such conditions, the liver functional tests remained within normal limits throughout the experiment, and it was possible to study the rate of some hepatic metabolizing activities, such as demethylation, acetylation, and glucurono-conjugation. In the course of systematic investigations on drug metabolism and tissue distribution (2–4) the research was extended to the metabolic activity of the brain, isolated *in situ* in the

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