mation was collected which indicates that the intradermal vaccinations had considerable effect. Thus, 7 of 166 participants (4%) who completed a clinical questionnaire had symptoms comparable with influenza. This is in contrast to 28 cases with similar symptoms among 199 non-vaccinated family contacts (14%). Ratio of the attack rates in non-vaccinated and vaccinated groups in the present study (3.7) is interestingly similar to the corresponding ratio (3.6) in the study of Sigurjonsson *et al.* (8) even though the attack rates in the latter were much higher.

Undesirable side reactions from vaccinations are factors which greatly affect acceptance of a vaccination program. Although the amount of vaccine injected affects reaction rate and severity of the reaction, other studies performed simultaneously with this study but using different vaccines clearly indicated that reaction rate and severity of reaction vary as much from vaccine lot to vaccine lot as they varied in this study from dose to dose.

Summary. The immune response elicited by 2 intradermal injections of 0.1 ml monovalent Asian A influenza vaccine administered 2 weeks apart was related to the antigenic potency of the vaccine. Individuals developing detectable antibodies 2 weeks after the second injection varied with dose from 44 to 70% with titers 1:40 or greater. Using the same lot and dosage schedule of vaccine an intradermal booster immunization administered 53 weeks after first vaccinations, induced marked rise in titers which were also related to dose. The geometric mean titers which ranged from 1:21 to 1:51 two weeks after second injection of vaccine rose to 1:51 to 1:211, 2 weeks after booster injection. In the present study, 2 separate injections of 50 CCA units resulted in better antibody responses than single injections of 100 CCA units. Even the response to 2 injections of 12.5 CCA units is as good as the response to single doses 4 times as large. Systemic and local reactions increased with unit dose of vaccine but no serious reactions occurred with the lot of vaccine used throughout this study.

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In vitro Metabolism of Mephenesin, 3-o-Tolyloxy-1,2-Propanediol. (25443)

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3-o-Tolyloxy-1,2-propanediol, mephenesin, a muscle relaxant, is characteried by an extremely short duration of action. Two metabolic products, 3-(o-tolyloxy)lactic acid (1,2) and 3 - (4-hydroxy-2 - methylphenoxy)lactic acid (3,4,5) have been identified in dog and human urine following administration of mephenesin. Aside from these *in vivo* observations, *in vitro* metabolism of this compound has not been reported beyond the brief comment of Maass, *et al.* (6). The metabolism of mephenesin by mouse and rat liver slices has

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TABLE I. Mephenesin Metabolism in 60 Min. by Rat and Mouse Liver Slices.

Animal	No. of exp.	Buffer	Mephenesin metabolized (by difference) μmole	μmole/g $\pm$ S.D.	3-(o-tolyloxy)lactic acid formed, μmoles
Rat	2	K.R. phosphate	3.41	7.91	2.94
Mouse	5	"	3.59	9.14 $\pm$ 2.63	1.63
Rat	2	K.R. bicarbonate	2.50	6.24	1.31
Mouse	5	"	5.64	14.58 $\pm$ 3.22	2.81

been studied under various experimental conditions and the principal metabolite has been identified and assayed.

Methods. Approximately 400 mg fresh weight of liver from adult rats or mice, usually 5 or 6 slices, were incubated with 11 μM mephenesin in 20 ml Krebs-Ringer (K.R.) phosphate (pH 7.4) or bicarbonate buffer (pH 7.4) in a 125 ml Warburg flask. The flasks were incubated for 30 to 180 minutes at 38°C with either 100% oxygen or 95% oxygen and 5% carbon dioxide. Phthalate, ethylene diamine, or borate buffers at pH 6.1, 7.4 and 8.1 were also tried but the reaction proceeded more rapidly in K.R. phosphate or bicarbonate buffer. The reaction was stopped by filtering the medium rapidly through Whatman No. 41 filter paper to separate medium and tissue. Trichloroacetic acid, which might have been used to stop the reaction, was found to interfere with the ultraviolet assay of the substrate and product, therefore, the filtration procedure was devised. After incubation the medium was routinely assayed for both mephenesin and 3-(o-tolyloxy)lactic acid(7). Mephenesin was exhaustively extracted from the incubation medium (pH 6.0-8.1) with ether. The medium was then adjusted to below pH 3.0 in order to suppress the ionization of the 3-(o-tolyloxy)lactic acid, which then was also readily extracted with ether. Substrate and product present were determined by recording the absorbance of the ether solutions between 240-300 mμ. The quantity of mephenesin or 3-(o-tolyloxy)lactic acid present was calculated from the absorbance at 270 mμ. The lack of absorbance at 290 mμ demonstrated the absence of any detectable quantity of 3-(4-hydroxy-2-methylphenoxy)lactic acid(4). To prove the identity of 3-(o-tolyloxy)lactic acid, the pooled ether extract was concentrated to 10 ml, then shaken gently with 10 ml of 0.1 N NaOH. Both layers were assayed spectrophotometrically at 300 and 270 mμ. Since 3-(o-tolyloxy)lactic acid is more readily soluble in NaOH than in ether, the presence of the characteristic ultraviolet absorption spectrum in the NaOH and the absence of absorption in the ether served to identify the reaction product. Mephenesin has a partition coefficient of 4.155 $\pm$ .093 in this system. Recovery of mephenesin, incubated with heat inactivated liver slices averaged 101.0% with a standard deviation of 5.7%. A small and variable amount (2.3 to 6.2%) of the mephenesin in the medium adhered to the tissue slice. Therefore, the tissue as well as the medium was assayed for mephenesin. Recovery of 3-(o-tolyloxy)lactic acid averaged 102.8 $\pm$ 3.2% in bicarbonate buffer and 106.1 $\pm$ 3.0% in phosphate buffer. There was no apparent tissue binding of the 3-(o-tolyloxy) lactic acid.

Results. A heat labile enzyme system present in rat or mouse liver slices rapidly oxidized mephenesin to 3-(o-tolyloxy)lactic acid (Table I and Fig. 1). Rat or mouse liver homogenates, supplemented with various cofactors under these conditions, did not cause oxidation of mephenesin. No metabolism occurred in presence of 100% nitrogen.

Representative data showing the relationship of mephenesin oxidation to tissue weight, substrate concentration and incubation time have been indicated in Figs. 1-3. The μmoles of mephenesin metabolized was directly related to amount of enzyme present (Fig. 1) but amount of 3-(o-tolyloxy)lactic acid formed was not proportional to tissue weight. The μmoles of mephenesin metabolized per gram of fresh tissue was apparently a linear relationship with time up to 60 minutes (Fig. 2).

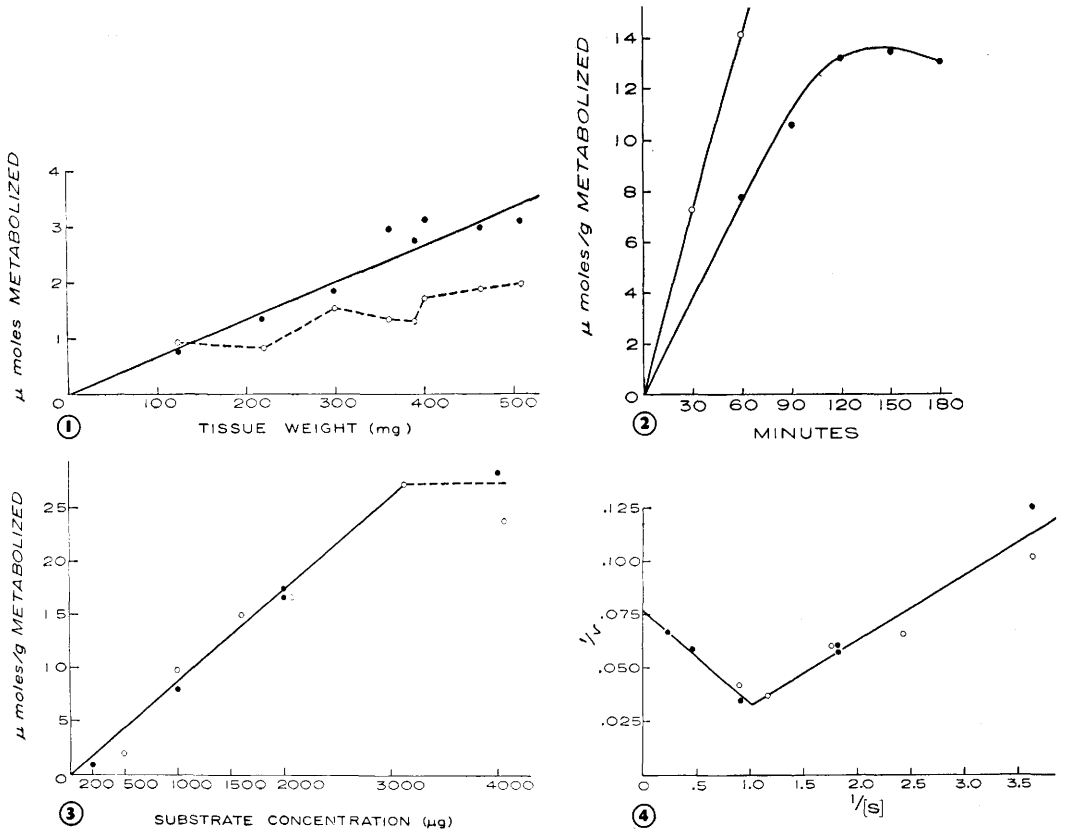

FIG. 1. μmoles of mephenesin oxidized (●) and 3-(o-tolyloxy)lactic acid (○) formed by increasing weight of mouse liver slices incubated in K.R. phosphate buffer (pH 7.4) for 60 min.

FIG. 2. μmoles of mephenesin oxidized by rat liver slices (●) in K.R. phosphate buffer (pH 7.4) or by mouse liver slices (○) in K.R. bicarbonate buffer (pH 7.4) between 30 and 180 min.

FIG. 3. Effect of substrate concentration on the rate of oxidation of mephenesin. Mouse liver slices incubated for 30 min. (○) or 60 min. (●) in K.R. bicarbonate buffer (pH 7.4).

FIG. 4. Effect of increasing substrate concentration [s] on observed velocity (v) of mephenesin oxidation. Mouse liver slices incubated for 30 min. (○) or 60 min. (●) in K.R. bicarbonate buffer (pH 7.4) containing 0.27 to 4.39 mM of mephenesin. Since reaction rate was linear for at least 60 min. the μmoles of mephenesin oxidized/g of fresh tissue/30 min. was doubled in calculating reciprocal of velocity.

The optimum substrate concentration was determined by incubating varying concentrations of mephenesin in bicarbonate buffer with approximately 400 mg of mouse liver slices (Fig. 3). The oxidation was depressed at low substrate concentrations, presumably due to a dilution effect. The maximum percent of substrate oxidized occurred between 1500-2000 μg. However, since rate of oxidation appeared to fall with increasing substrate concentrations, the data were extended and a Lineweaver and Burk(8) plot of the data (Fig. 4) clearly indicated inhibition of oxidation at concentrations above 200 γ/ml suggesting that the enzyme has 2 points of attachment for the substrate. It is obvious that mephenesin was not completely converted to 3-(o-tolyloxy)lactic acid (Table I). This suggested that either the 3-(o-tolyloxy)lactic acid was being further metabolized or that an intermediate metabolite between the alcohol and the acid was being formed. Although a number of conditions were tried none were found which would force the metabolism of 3-(o-tolyloxy)lactic acid. Addition of increasing amounts of 3-(o-tolyloxy)lactic acid to the medium actively oxidizing mephenesin had only a slight effect on rate of mephenesin metabolism (Table II). However, since there was a marked reduction in amount of 3-(o-

TABLE II. Effect of Increasing Amounts of 3-(o-tolyloxy)lactic Acid on Metabolism of Mephenesin.

3-(o-tolyloxy)-lactic acid, μmoles added	Mephenesin metabolized* (by difference) μmoles	μmoles/g	3-(o-tolyloxy)-lactic acid formed, μmoles
0	5.88	16.59	3.40
1.61	5.76	16.07	3.23
5.29	6.15	16.14	2.47
10.47	5.65	15.21	1.58
20.93	5.80	14.79	.13
41.88	5.36	14.29	.0

* K.R. bicarbonate buffer, 60 min. incubation.

tolyloxy)lactic acid formed, these data suggested that an intermediate metabolite was formed and that 3-(o-tolyloxy)lactic acid was not the immediate but the final product of mephenesin metabolism.

Since an aldehyde seemed to be the most likely intermediate, mouse liver slices were incubated with aldehyde trapping agents. Addition of either sodium bisulfite, hydroxylamine or semicarbazide at 10^{-4} and 10^{-3} M concentrations, which had no effect on oxygen consumption of the slices, had no effect on rate or extent of mephenesin oxidation, nor did they significantly alter amount of 3-(o-tolyloxy)lactic acid formed.

Alcohol and aldehyde dehydrogenases have been shown to require intact sulfhydryl groups for activity. Addition of iodoacetic acid to the medium indicated that mephenesin metabolism was sensitive to sulfhydryl inhibitors, for the oxidation was completely inhibited by concentrations as low as 3 mM. Preliminary studies indicated that mephenesin was not metabolized by mouse diaphragm, heart, spleen, or brain slices. Kidney slices oxidized mephenesin slowly.

Berger(9) reported that the LD_{50} for mephenesin, administered intravenously, increased from 195 mg/kg for the rat, to 220 mg/kg for the rabbit, to 322 mg/kg for the mouse. These data suggest that the mouse metabolizes the drug more rapidly than the rat. Our observations on the rapidity of mephenesin oxidation by the rat and the mouse support this conclusion.

Summary. 1. Mephenesin is oxidatively metabolized *in vitro* by rat and mouse liver slices to 3-(o-tolyloxy)lactic acid. 2. Mephenesin metabolism was markedly inhibited by increasing substrate concentration, but was not altered by presence of increasing concentrations of the product, 3-(o-tolyloxy)lactic acid, in the medium. 3. Metabolism of mephenesin was readily inhibited by iodoacetic acid, but was unaltered by aldehyde trapping agents at 10^{-3} M.

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Preparation of Formalinized Erythrocytes. (25444)

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Following discovery by Flick(1) that red blood cells treated with formaldehyde could be preserved for long periods of time, several investigators(2-4) devised methods of preparing formalinized cell reagents in which special properties of red cells were utilized(5). We prepared formalinized red cells by published methods which were not satisfactory for use in agglutination tests by the pattern method (6), because of crenation, inseparable clumps, and non-specific aggregation. Then we devised a method that permitted us to prepare con-