mary tissue. Griffith and Turner (unpublished results) have recently observed an increase in total DNA of pregnant rats injected daily with 2.5 or 3.5 μg/100 g thyroxine. It has been shown also that pregnant rats fed desiccated thyroid exhibit precocious mammary gland development(10). These data indicate that during pregnancy endogenous secretion of E and P may be sufficient to overcome the increased metabolic removal of the steroids during hyperthyroidism and as a result, mammary gland growth may be increased. Although the thyroid hormone is not essential for mammary gland growth(11), the present data suggest that variability in total mammary gland growth may be due to a deficiency in TSR or of E and P, or both. When the TSR is high, it is fully effective in stimulating the greatest mammary gland growth only when endogenous E and P secretion is adequate.

Summary. 1) The effect of mild hyperthyroidism upon mammary gland growth has been studied in adult ovariectomized rats treated with estradiol benzoate (EB) and progesterone (P) for 19 days. 2) Using total DNA as index of mammary growth, glands of animals receiving daily injections of 1 μg EB, 3 mg P and 3 or 6 μg/100 g thyroxine did not differ from that of controls. Upon doubling EB and P levels, a significant increase in total DNA was observed. 3) It is suggested that thyroid hormone may be a limiting factor in mammary gland growth when E and P secretions are adequate, but when TSR is optimal, E and P secretion may limit growth.

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Intradermal Vaccination in Healthy Adults With Type A Asian Strain Influenza Vaccine.* (25442)

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The intradermal route for administration of Asian strain Type A influenza vaccine was employed widely by practicing physicians in the fall of 1957 when the demand for vaccine exceeded the supply. In view of widespread use of this method of vaccination, this study was undertaken to determine the relative efficiency of various doses of vaccine in producing measurable immune responses. In Sept. 1957 when the program was started, no clinical influenza had been reported in the study area. The vaccine used throughout was a single lot of commercially prepared monovalent Type A Asian strain influenza vaccine which contained 1000 chicken cell agglutination (CCA) units/ml. To administer different amounts of vaccine in the same volume the vaccine was diluted with pyrogen-free saline to make 4 concentrations containing 12.5, 25, 50, and 100 CCA units in 0.1 ml. Two hundred and forty-two civilian employees from

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the Army Corps of Engineers in Atlanta between ages 20 and 60 volunteered for the study. These individuals were randomly assigned to 5 groups which received 0.1 ml vaccine intradermally in the volar surface of the forearm, (Table I). Groups I-IV received a second dose of vaccine after a 2-week interval; Group V received only one injection. In Oct. 1958, 53 weeks after initial program was begun, a booster vaccination was administered to 79 volunteers. The booster dose for each was the same size as the first dose. Venous blood was collected from all volunteers prior to administration of the first injection of vaccine (week 0), prior to second dose (week 2) and again 2 weeks later (week 4). Additional blood specimens were obtained from 75 individuals 16 weeks after first injection. During the booster phase of the program blood specimens were collected from all returning individuals just prior to vaccine injection (week 53) and again 2 weeks later (week 55).

Results. Antibody response. Antibody titers were determined by the hemagglutination inhibition test using the A2/Japan/305/57 antigen (EFME)(1,2). Antibody titrations were done by using 2-fold serum dilutions starting at 1:10. For calculation of geometric mean titers, actual titers were used for those classed as 320-1280 in Table I. Two weeks after the first injection of vaccine, there was slight but definite antibody response increasing with dose. This ranged from 15% in Group I to 47% in Group IV at 1:20 (Table I). Two weeks after the second vaccination, the percentage of individuals with antibody titers 1:20 or greater had further increased in all study groups to a range of 63 to 90% including Group V which had received only one dose. However, 16 weeks after the first dose when 75 individuals returned for blood sampling, 26 individuals (35%) with measurable antibodies at week 4 (highest titer 1:320) had lost all their detectable serum antibodies and 45 individuals (60%) had up to 16-fold decreases of titers and only 4 individuals (5%) had maintained their titers. The geometric mean titers of different groups stayed approximately on the same level between weeks 16 and 53. The booster dose in each group resulted in pronounced titer increases. (Table I).

The number of individuals decreased as the study progressed. The individuals who participated in the entire study produced titers in the initial vaccination program which did not vary significantly from the total group and therefore were considered as a representative sample.

Reactions to vaccine. No individuals with a history of sensitivity to eggs were included in this study. The individuals included did not know what dose of vaccine they were receiving. The sites of vaccine injections were observed 24 hours after first and second vaccination. An increasing erythema was noted with increased dosage, but there was no significant difference in the local reaction after the first and second injections. The mean diameter of erythematous areas was 17 mm in Group I; 20 mm in Group II; 26 mm in Group III; and 38 mm in Groups IV and V.

Careful questioning of each individual showed that greater doses caused more subjective symptoms than the smaller doses. The proportion of individuals with systemic complaints after the first injection of vaccine ranged from less than 10% in Group I to over 50% in Groups IV and V. The most frequent complaints were headaches, slight generalized muscular aches, drowsiness, dizziness, nasal congestion, chilly sensations and flushing. A few reported discomfort at site of injection. Three per cent of those in Groups I and II and 15% in groups III, IV, and V reported fever 6-24 hours after first injection. After the second injection of vaccine less generalized symptoms were reported, and still less after the booster injection. No generalized urticaria or asthma were reported.

Discussion. It is not possible to determine on the basis of this study how the antigenic effect of intradermal administration of influenza vaccine compares with subcutaneous or intramuscular injection of the same vaccine. However, one may note that the circulating antibody response to intradermal immunization in this study compares quite favorably with several other studies in which varying lots of Asian influenza vaccine were administered subcutaneously(3,4,5,6).

TABLE I. Titer Distribution Following Varying Doses of Vaccine by Intradermal Route.

Dose in CCA units	Wk after vaccination	No. of individuals								Geometric mean titers*	
		Total	<10	10	20	40	80	160	320-1280	1	2
Group I											
12.5	0	52	51	1	0	0	0	0	0	<10	<10
"	2	52	32	12	3	2	2	1	0	<10	<10
	4	39	7	7	8	12	4	1	0	21	20
	16	19	15	2	1	1	0	0	0	10	10
"	53	18	13	2	0	2	1	0	0	10	10
	55	18	0	0	5	5	3	5	0	54	54
Group II											
25.0	0	47	47	0	0	0	0	0	0	<10	<10
"	2	47	25	6	8	4	3	0	1	11	<10
	4	33	7	5	6	8	2	4	1	24	34
	16	10	9	0	0	1	0	0	0	10	10
"	53	11	7	3	0	0	1	0	0	10	10
	55	11	1	0	1	5	1	2	1	51	51
Group III											
50.0	0	50	49	1	0	0	0	0	0	<10	<10
"	2	50	26	5	11	3	4	1	0	11	11
	4	25	3	1	6	3	8	2	2	41	40
	16	14	10	2	0	2	0	0	0	10	10
"	53	15	11	1	1	1	1	0	0	10	10
	55	15	0	0	0	4	6	3	2	100	100
Group IV											
100.0	0	93	93	0	0	0	0	0	0	<10	<10
"	2	93	36	13	15	12	9	2	6	17	21
	4	20	1	1	4	6	3	2	3	51	69
	16	14	5	5	2	0	0	1	1	13	16
"	53	10	4	2	1	1	1	1	0	15	15
	55	10	0	0	0	2	0	4	4	211	211
Group V											
100.0	0	93	93	0	0	0	0	0	0	<10	<10
	2	93	36	13	15	12	9	2	6	17	16
	4	48	11	7	13	7	5	1	4	22	26
	16	18	10	2	0	3	1	2	0	13	14
"	53	25	11	4	6	2	2	0	0	11	11
	55	25	0	0	1	6	7	8	3	100	100

* 1—Includes all sera tested. 2—Includes only those individuals who returned for 53 and 55 wk blood tests.

It is probably impossible to establish any single level of circulating antibody which would represent an adequate protective titer against "natural infection." In the study of Bell and associates(7) in which vaccinated and non-vaccinated individuals were challenged with live virus, there was evidence of vaccine protection in those who had developed detectable antibody titers 1:10 or greater, following vaccine. The authors of this paper measured the circulating antibody level of 65 nonimmunized convalescents approximately 3 weeks after illness. In this group 82% of the convalescents had titers 1:20 or higher and 52% 1:40 or higher. The geometric mean titer of the convalescent group was 1:29. These antibody values compare closely with those in Groups II and III in the present study.

If the circulating antibody responses in different vaccination groups are compared, one will note that 2 doses of 50 CCA units at 2 week intervals gave better antibody response than a single dose of 100 CCA units and even 2 doses of 12.5 CCA units of vaccine resulted in a response comparable to a single dose containing 4 times as much total antigen.

Attempts were made to determine the protective value of the vaccination during the epidemic which followed in Atlanta several weeks after immunizations were completed. Although follow-up studies could not be performed on the total number of cases scattered throughout the metropolitan area, some infor-

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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