TABLE I.

Granulation tissue	Titre in units/g
A. Tissue from experimental granulating canine wounds	
1. Dog. No. 1 Age of wound	
3 days	80
8 "	108
14 "	208
2. Dog No. 2	
a. from center of wound, 14 days	77
b. from periphery—mostly new epithelium, 14 days	10
B. Tissue from granulating human wounds	
1. Indolent ulcer from pyoderma gangrenosum in chronic ulcerative colitis; 2 occasions	23; 30
†2. Same wound after healing had begun (chemotherapy)	170
†3. Granulating dog bite in a human (1 week)	224
4. Indolent varicose ulcer	91
5. " " "	81
†6. Granulating thoracotomy wound	564
†7. Granulating laparotomy wound	161
8. Necrotic granulations from laparotomy wound	500
†9. Infected laparotomy wound	91
†10. " " "	118
†11. " " "	159
C. Rabbit callus determinations in fractured femur	
1. 5 days	84
2. 8 "	60
3. 14 "	16

† Healthy appearing granulating tissue.

ulation tissue was therefore unexpected. The lysozyme assays on granulation tissue of man and dog are shown in the accompanying table.

The assays were done by a viscosimetric method[4] on extracts prepared as previously described.[3]

As a result of these observations it is now apparent that high lysozyme concentrations are associated with some mesodermal cell types as well as with epithelium. Therefore, further study is warranted with regard to the role of this tissue in the production of lysozyme in ulcerative alimentary disease.

The deleterious effect of egg white lysozyme on the gastrointestinal mucosa[1,3] has been confirmed in our own[5] as well as other laboratories.[6,7] Furthermore, high stool titres in the absence of occult blood in the feces and with sigmoidoscopically non-ulcerated mucosa are frequently observed in chronic ulcerative colitis. These two considerations render less likely the possibility that granulation tissue is the source of the major fraction of the lysozyme titre in ulcerative alimentary disease.

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Occurrence of Conjugase in Egg Yolks.* (17325)

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Pteroylglutamic acid conjugases are enzymes that liberate microbiologically available PGA from more complex forms of the vitamin and have been reported in hog kidney,[1] chicken pancreas,[2,3] rat liver[4] and blood.[5] During the course of routine PGA determina-

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TABLE I.
Pteroylglutamic Acid Values Obtained by Various Treatments of Egg Yolk.*

Treatment of sample	Autoclaved†	Not autoclaved	Autoclaved + chick pancreas	Not autoclaved + chick pancreas	Not autoclaved + autoclaved chick pancreas
	Micrograms of PGA per 100 g of egg yolk (fresh weight basis)				
Chicken egg yolk	10.2	31.9	35.3	81.4	16.3
Turkey egg yolk	41.9	60.2	62.9	129.7	60.8

* Each value in this table is the average of seven separate assays.
† All samples were incubated at 37.5° C for 24 hours.

tions in the laboratory, an indication was obtained that egg yolk also contains such a conjugase and the present study was initiated to determine if this was correct.

Experimental. In order to test this hypothesis, the following treatments of samples were used. In the first place the sample was autoclaved for 15 minutes at 15 lb pressure, incubated for 24 hours at 37.5°C and assayed for free PGA. In the second treatment the sample was not autoclaved but was incubated in an effort to determine if any conjugase was present in the egg yolk. In the third treatment the sample was autoclaved and 20 mg of dried chick pancreas was added prior to incubation of the sample, in order to ascertain the amount of PGA liberated by the conjugase of chick pancreas. The fourth treatment consisted of adding chick pancreas to the unautoclaved egg yolk sample in order to determine total PGA and finally 20 mg of autoclaved chick pancreas was added to a sample of egg which had not been autoclaved in order to determine if egg yolk conjugase would liberate PGA from chick pancreas. The tentative assay procedure for PGA, as devised by Flynn[6] for AOAC collaborators, was used in determining the microbiologically available PGA with *Lactobacillus casei* as the test organism; growth was measured by acidimetry. According to this procedure the sample is autoclaved first and then chicken pancreas is added prior to incubation.

Chicken egg yolks used in these studies were taken from eggs laid by Single Comb White Leghorn pullets that had been in production for approximately 4 months. The turkey egg yolks were obtained from eggs laid by Broadbreasted Bronze turkey pullets that were just beginning to lay. Both the turkeys and chickens were fed practical all-mash diets and were maintained in individual laying cages with raised screen bottoms. All eggs were collected daily and placed in a refrigerator at a temperature of approximately 4.4°C. In each case the pteroylglutamic acid determination was carried out on an individual egg yolk. The egg was broken and the white was separated and discarded. A 10 g sample of the fresh yolk was blended in the Waring blendor with 90 ml of distilled water. A 10 ml aliquot was pipetted into a 125 ml Erlenmeyer flask. Thirty ml of phosphate buffer (pH 7.0) and 10 ml of a chick pancreas solution, which contained 2 mg dried chick pancreas per ml, were added. The dried chick pancreas was prepared according to the method of Burkholder, McVeigh and Wilson.[7] The treatment of the samples prior to incubation is indicated in Table I. All samples were covered with a layer of toluene and incubated at 37.5°C for 24 hours. After incubation the samples were autoclaved for 15 minutes at 15 lb pressure, diluted to 100 ml, filtered and aliquots were taken from this stock solution for dilution purposes.

When the egg yolk sample was autoclaved for 15 minutes at 15 lb pressure and incubated it was assumed that any enzymes which

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TABLE II.
Liberation of PGA from Egg Yolk by Different Conjugases.

Liberation by different conjugases	Free PGA	PGA liberated by egg conjugase	PGA liberated by chick pancreas conjugase	Total PGA	PGA liberated by synergistic action of egg yolk and chick pancreas conjugase	PGA liberated by egg conjugase from chick pancreas
	Micrograms of PGA per 100 g of egg yolk (fresh weight basis)					
Chicken egg yolk	10.2	21.7	25.1	81.4	24.4	—15.6
Turkey egg yolk	41.9	18.3	21.0	129.7	48.5	+0.6

were present were destroyed. Free PGA determined on such a sample amounted to 10.2 μg per 100 g of chicken egg yolk (Tables I and II). The free PGA value for turkey egg yolk was about 4 times that of the chicken egg yolk.

The incubation of samples of egg yolk that had not been autoclaved resulted in an increase in PGA over that of the autoclaved sample described above (Tables I and II). The magnitude of this increase was about the same for both the chicken and turkey egg yolks and amounted to 21.7 μg PGA per 100 g yolk in the chicken egg and to 18.3 μg per 100 g in the turkey egg yolk (Table II). From these results it is apparent that egg yolk from the chickens and turkey contains a conjugase which liberates microbiologically available PGA.

When the sample was autoclaved and chick pancreas added as outlined in Flynn's procedure[6] this source of conjugase liberated 21.0 μg of PGA per 100 g of chicken egg yolk and 25.1 μg of PGA per 100 g of turkey egg yolk (Table II). These values are increases above the free PGA values and must have resulted through the action of chicken pancreas conjugase. The value obtained for chicken egg yolk where the sample was autoclaved and chick pancreas added (Table I) is in the range of that obtained by the AOAC collaborators (Flynn[6]) when it is converted to a dry weight basis.

The total PGA, determined by adding 20 mg of chick pancreas to the unautoclaved sample, was 81.0 μg per 100 g for the chicken egg yolk and 129.7 μg PGA per 100 g of turkey egg yolk. These figures are higher than that which can be calculated by adding free PGA, PGA liberated by egg yolk conjugase and that liberated by chick pancreas conjugase (Table II). A total of these values gives 57.0 (10.2 + 21.7 + 25.1) μg PGA per 100 g chicken egg yolk and 81.2 (41.9 + 18.3 + 21.0) μg per 100 g turkey egg yolk. Thus 24.4 and 48.5 μg PGA per 100 g egg yolk (Table II) were liberated by the synergistic action of egg yolk and chicken pancreas conjugases for the chicken and turkey egg yolks respectively. The total PGA value obtained for chicken egg yolk when converted to the dry weight basis approaches the lower value for PGA obtained by Flynn and collaborators[6] when dried egg yolk was assayed for PGA by the chick assay. Thus with the combined action of egg yolk conjugase and chick pancreas conjugase the value for the microbiologically available PGA approaches a similar value obtained by means of the chick assay for this vitamin.

One sample was tested in each series to determine whether egg yolk conjugase was liberating PGA from the chick pancreas. In this instance the chick pancreas solution (2 mg per ml) was autoclaved for 15 minutes at 15 lb pressure and added to the unautoclaved egg yolk. The results (Table I) show that no appreciable liberation of PGA from chick pancreas was obtained by turkey egg yolk conjugase. A slight inhibitory effect was noted on the conjugase in chicken egg yolk when autoclaved chick pancreas was added prior to incubation. The PGA liberated by egg yolk conjugase amounted to only 6.1 μg per 100 g; whereas, 21.7 μg PGA was liberated by egg yolk conjugase when autoclaved chick pancreas was not added. This represents a decrease of 15.6 μg PGA per 100 g yolk in

the presence of autoclaved chick pancreas (Table II).

From the results of this study maximum liberation of PGA from egg yolk occurs when egg yolk conjugase and chick pancreas conjugase act together.

Summary. Evidence is presented to show that chicken and turkey egg yolk contain a conjugase which liberates PGA from these materials. A synergistic action of egg yolk conjugase and chick pancreas is required in order to obtain the total PGA in egg yolk of chickens and turkeys. The liberation of microbiologically available PGA from egg yolk by the combined action of egg yolk conjugase and chicken pancreas conjugase gives a total PGA value which approaches the value obtained by the chick assay for this vitamin. An inhibitory effect of autoclaved chick pancreas on the conjugase of chicken egg yolk is indicated by the results.

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Effect of Thyroxine on Lymphoid Tissue Mass of Adult Male Mice.* (17326)

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That the secretion of the thyroid gland is intimately related to the physiology of lymphoid tissue has been suggested by both clinical observation and laboratory experiment. Marine and co-workers[1] emphasized that peripheral lymphadenopathy, splenomegaly, and persistence of the thymus gland were detectable in many patients with Graves' disease. Several investigators[2-4] have reported that stimuli which cause accidental involution of the lymphoid tissue of laboratory animals may have a more pronounced or more prolonged effect in the absence of the thyroid gland.

An important link in the chain of evidence —namely that augmented thyroid secretion results in hyperplasia of lymphoid tissue— has previously evaded demonstration despite several experimental trials.[3,5] One possible explanation for this failure lies in the fact that the rat was the experimental animal employed in most of these investigations. Evidence is accumulating that this animal, in the resting state, may be secreting thyroid hormone at a high rate;[6] thus the addition of small doses of exogenous hormone might well be without significant effect. Large doses of hormone may produce relative malnutrition and involution, rather than hyperplasia, of lymphoid tissue.[7] It was, therefore, considered desirable to observe the consequences of thyroxine treatment in the mouse. Profound changes in the lymphoid tissues were observed. In view of the known alterations in adrenal cortical activity following treatment with thyroid hormone,[8,9] a parallel experiment was conducted in adrenalectomized animals to study the possible contribution of this organ

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