or handling. All these factors must be considered before conclusions are drawn.

Summary. When irradiated dogs developed severe thrombocytopenia and purpura, the thoracic duct was cannulated and output of red cells/minute counted to quantitate bleeding tendency. Fresh canine platelets disintegrated in a sonic oscillator and infused into the dogs increased prothrombin consumption towards the normal range, but had no effect on bleeding tendency. Intact platelets, however, had an immediate and dramatic effect on bleeding tendency. We conclude that a normal clotting system, including platelet factors, is not enough for normal hemostasis; the mechanical effect of intact platelets is also needed. Consequently, non-intact platelets or platelet substitutes may not be expected to improve the bleeding tendency in thrombocytopenia.

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Improvement in Nutritional Value of Peas by Cooking.* (25133)

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Numerous attempts have been made to improve the biological value of field peas (*Pisum sativum*). Woods *et al.*(1) demonstrated that protein of field peas is deficient in methionine, and that baking and autoclaving decreased growth promoting properties of the protein for rats. Peterson *et al.*(2) showed that chick growth was improved by adding methionine to diets containing peas. Our preliminary results indicated that diets containing large percentages of peas would not support maximum chick growth when methionine deficiency was corrected. Therefore, studies were undertaken to determine the influence of different methods of treating peas on growth of chicks and poults.

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Methods. Mature Alaska peas (*Pisum sativum*) grown in Eastern Washington were used. Cooking was accomplished by mixing ground peas with 1 to 2 parts water (approximately 30°C), spreading approximately one inch deep on trays, and autoclaving at various times and pressures (Table I). Cooked peas were immediately cooled before a fan, then cut into ½″ squares and dried in forced draft oven at 60°C. Peas were stirred after 4 hours to increase drying rate and removed from oven after 24 hours. They were then re-ground through a ⅛″ screen, and mixed in the diets. Uncooked frozen peas (var. Thomas Laxton) were partially dried in air blast, then placed in oven at 60°C to complete drying. Autoclaved peas (Table III) were prepared in same manner as cooked peas, except water

TABLE I. Effect of Cooking Peas on Poult Growth and Feed Efficiency.

Type of peas	Cooking time (min.)	Steam pressure (p.s.i.)	Ratio by wt of added H_2O:peas	Avg wt* 2 wk (g)	Feed / Gain
Corn-soy diet (control)				231b $\alpha\beta$	1.30
Mature Alaska	0	0		147e $\delta\epsilon$	1.91
Idem	0	0	1 :1	165d δ	1.69
"	15	10	1 :1	203c γ	1.43
"	15	10	1½:1	210c $\beta\gamma$	1.44
"	15	10	2 :1	211c $\beta\gamma$	1.39
"	30	10	1 :1	198c γ	1.42
"	30	10	1½:1	237ab α	1.47
"	30	10	2 :1	233b $\alpha\beta$	1.35
Mature Thomas Laxton (dry)	0	0	0	135e ϵ	2.10
Immature Thomas Laxton (fresh frozen)	0	0		205c γ	1.61
Idem	30	5		252a α	1.34

* Latin letters after weight in this column refer to significance at 5% level, and Greek letters refer to significance at 1% level. Treatments with a common letter are not significantly different at level indicated. Treatments without a common letter are significantly different at level indicated.

was not added. Day-old Broad Breasted Bronze poults or New Hampshire chicks were randomly distributed into pens of 10 birds each and given feed and water *ad lib*. Poults and chicks were maintained in electrically heated batteries with raised wire floors. Each experimental diet was fed to 3 groups of birds. A 28% protein basal poult diet of following percentage compositions was used: 77.10 peas (24% protein), 12 herring fishmeal, 3 dehydrated alfalfa meal, 4 dicalcium phosphate, 2 limestone, .5 salt, .6 DL-methionine, and vitamin mix supplying 5300 I.U. Vit. A, 1100 I.C.U. Vit. D, 6.6 mg riboflavin, 15.4 mg pantothenic acid, 1.65 g choline chloride, .11 mg folic acid, 22 mg niacin, 11 mg procaine penicillin, 11 I.U. Vit. E, and .2 g manganese sulfate/kilo of diet. The control diet was 48.6% corn and 28.5% dehulled soybean oil meal, replacing peas. A 23.4% protein basal chick diet of following percentage composition was used: 93.4 peas (24% protein), 2 tallow, 1.5 steamed bonemeal, 1.5 limestone, .5 salt, 16 DL-methionine, .02 manganese sulfate and vitamin mix supplying 2640 I.U. Vit. A, 440 I.C.U. Vit. D, 2.86 mg riboflavin, 9.24 mg pantothenic acid, 880 mg choline chloride, 11 mg procaine penicillin, 26.4 mg niacin, 2.86 mg pyridoxin-HCl, .088 mg biotin, .0088 mg Vit. B_{12}, .55 mg folic acid, 1.76 mg thiamine-HCl, 22 I.U. Vit. E, and .396 mg menadione bisulfite/kilo of diet. Control diet was 56.8% corn and 36.6% dehulled soybean oil meal, replacing peas. Separation of peas into 2 fractions (Table IV) was accomplished by stirring 1 kilo of finely powdered peas into 5 kilo of 0.6% NaOH, adjusting to pH 11.3 and centrifuging. The solid residue (alkali-insoluble peas) was air dried with fans and ground before mixing into diets. The alkali-soluble portion was adjusted to pH 5.5 with concentrated HCl and centrifuged. The precipitate (alkali-soluble peas) was treated as above before mixing into diets. Alkali-soluble fraction contained 64% and the alkali-insoluble fraction contained 7% protein (Kjeldahl).

Results. Results of 4 experiments are reported. Cooking of both mature Alaska peas and fresh frozen Thomas Laxton peas markedly improved growth rate and efficiency of feed utilization in turkeys (Table I). A greater improvement was obtained by cooking peas 30 minutes as contrasted to 15 minutes, and by using a ratio of water to peas of 1½ : 1 and higher. Results comparable to control diet were obtained by cooking peas 30 minutes with higher water-to-peas ratio. Immature peas (var. Thomas Laxton) were much superior to mature peas when compared on uncooked basis.

Growth, feed efficiency, and dry matter retention (determined by difference between dry matter ingested and dry matter eliminated) of chicks were markedly improved by

TABLE II. Effect of Cooking Peas and of Added Tallow on Performance of Chicks.

Tallow (%)	Peas in diet (%) Raw	Cooked	Soybean oil meal (%)	Productive energy (cal/g)	Avg wt 4 wk (g)	Feed / Gain	Dry matter retention 4th wk (%)
Corn-soy control diet				2.16	347	1.87	70
2	93.4			1.96	227	2.66	55
2		93.4		2.02*	366	2.00	64
5	85.0		6	2.07	238	2.10	
5		85.0	6	2.12	340	1.77	
20	52.0		24	2.56	289	1.82	

* Fraps' productive energy value for cooked peas is 1.917 cal/g, and for raw peas is 1.954 cal/g.

cooking peas (Table II). Although increasing the productive energy content of diets by adding tallow brought about some improvement in growth and feed efficiency, the improvement was not as great as by simply cooking the peas. Autoclaving peas also improved both the growth and efficiency of feed utilization in chicks (Table III). Diet containing alkali-soluble fraction supported significantly ($P<.01$) better growth than diet containing alkali-insoluble fraction. Raw pea protein readily extracted by dilute alkali was high in feeding value.

The reason for nutritional improvement of peas by autoclaving is unknown. That it is not simply a physical change in peas, making more energy available to the animal, or improving protein quality, is shown by results presented in Tables II and IV, respectively. Increasing the caloric content of pea diets by addition of tallow did not give as great a response as the cooking treatment, even though calculated energy content of the diet was greatly increased.

Perhaps the best explanation is that heat treatment inactivates a growth inhibitor or toxic substance in peas. Several growth inhibitors for chicks have been demonstrated in various plant materials(3,4,5,6). One of these, a trypsin inhibitor found in raw beans, is inactivated by heating in presence of moisture. Borchers *et al.*(7), however, did not detect trypsin inhibitor in peas. Another member of the pea family, *Lathryus odoratus,* contains a toxic substance for animals, B(r-L-glutamyl) aminopropionitrile(8), which was not inactivated by cooking(9) or heating(10).

Armbruster and Murray(11) observed that cooked peas were not superior to raw peas for weanling rats. Our recent experiments showed that cooking peas slightly depressed female rat growth, but significantly ($P<.01$) increased male rat growth. No such difference between sexes have been noted with chicks or poults fed cooked peas. The reason for differences in response of chicks and rats to cooking of peas is not known.

TABLE III. Effect of Autoclaving of Peas *(Pisum sativum)* on Growth of Chicks.

Diet	Avg wt* 3 wk (g)	Feed / Gain
Corn-soy (control diet)	223a α	1.72
Raw peas	161b β	2.30
Cooked peas, 30 min.	230a α	1.87
Autoclaved, 30 "	229a α	1.86

* See footnote, Table I, for explanation of letters in column.

TABLE IV. Effect of Alkali-Soluble and Insoluble Fractions of Peas on Chick Growth.

Diet	Avg wt* 3 wk (g)	Feed / Gain
Positive control (corn-soy)	213b α	1.77
Raw peas	148d γ	2.66
Autoclaved peas	227ab α	1.81
Alkali-soluble peas†	231a α	1.79
" -insoluble " ‡	185c β	1.94

* See footnote, Table I, for explanation of letters in column.

† Contained 16.4% alkali-soluble fraction (64% protein), and 26.12% sucrose replacing autoclaved peas in autoclaved pea diet.

‡ Contained 74.4% alkali-insoluble pea fraction (7% protein) and 19.0% isolated soybean protein (Archer-Daniels-Midland Assay C-1), replacing 93.4% peas in diet.

Summary. Growth and feed utilization of chicks and poults fed diets containing high levels of peas (*P. sativum*) were markedly improved by cooking or autoclaving peas prior to mixing into diets. Greatly increasing cal-

oric content of diets by adding tallow did not give a growth response comparable to that obtained by simply cooking the peas. Alkali-insoluble fractions of peas depressed chick growth but alkali-soluble fractions did not. It is suggested that peas (*P. sativum*) contain a growth inhibitor for birds that is inactivated by heating treatments employed.

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Electrophoretic Migration of Serum Lipoproteins in Starch Gel.* (25134)

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Electrophoresis in starch gel(1), is being increasingly used mainly because of its good resolution of protein fractions. During our studies of serum and cerebrospinal fluid proteins(2) with this method we found it advantageous to stain half of the gel electrophoretogram for lipoproteins. Oil red O was selected as coloring agent in staining the lipoproteins in starch gel as described by Smithies (personal communication). Initially considerable difficulties were encountered with reproducibility of the pattern using this method; later, however, we determined that these variations were mostly produced by inconstant composition of commercial oil red O which contains components that stain albumin and globulins as well as lipids. Partial purification has reduced these inconsistencies and has enabled relatively constant lipoprotein patterns in the starch gel to be obtained. To interpret the patterns obtained with oil red O, we thought it important to identify the bands that were seen. Serum lipoproteins were fractionated by starch granule electrophoresis and by sedimentation in the preparative ultracentrifuge. The isolated fractions were submitted to electrophoresis in starch gel and the results described below.

Materials and methods. Human serum was separated by centrifugation, pooled and stored at 4°C no longer than 48 hours before ultracentrifugation or electrophoresis. All chemicals were of reagent grade and the solvents were redistilled. Double glass-distilled water was used in all experiments to reduce contact of lipoproteins with copper ions. Starch gel electrophoresis was carried out as described by Smithies(1). Coloring of lipoproteins with oil red O was performed as previously reported(2) and crude oil red O was partially purified eliminating the protein staining components with a technic we developed(3). Spinco Model L preparative ultracentrifuge was used to prepare high and low density lipo-

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