when oxalated (or citrated) normal human plasma is added to equal volumes of thromboplastin and calcium, and is said to be equivalent to 100% prothrombin activity. This does not mean, however, that the prothrombin concentration in such a clotting mixture is 100%, since it is obvious that the test itself involves diluting the plasma threefold, and this, added to the dilution introduced by the anticoagulant, gives a clotting mixture with an actual plasma concentration of about 26%. But, since both unknown and normal control plasmas are similarly diluted, it is proper to express the findings in the unknown plasma in terms of per cent of activity of the control, but incorrect to speak of them as expressing concentration of prothrombin. Prothrombin activity as currently measured is a function not only of the amount of prothrombin present but also of accompanying inhibitors and accelerators which, directly or indirectly, affect its conversion rate. Heretofore, only platelets, plasma and serum accelerators have been generally recognized to influence prothrombin conversion rate. The role of natural inhibitors in the plasma was poorly appreciated principally because, as shown above, these inhibitors were placed at a disadvantage by the use of diluted plasmas and strong heterologous thromboplastins, in tests for prothrombin activity. It may be assumed that much information may be gathered regarding the role of these inhibitors in regulating prothrombin conversion, by utilizing clotting systems with high plasma concentration, suitable contacting surfaces and moderately strong homologous thromboplastins as activating agents(18).

Summary. 1. The response of varying concentrations of stable normal plasma to strong homologous thromboplastin may be expressed by a curve of the parabolic type. Clotting mixtures of high concentration resist activation by thromboplastin, an effect which is more evident with abnormally stable plasmas like those from hemophilics. 2. All current one-stage methods for determining prothrombin activity are done on diluted plasma. The actual plasma concentration in the Quick one-stage procedure is about 26%. 3. In clotting mixtures with a high plasma concentration the action of thromboplastin is resisted, prothrombin conversion is delayed and the rate of clotting of such plasmas cannot be used as an indication of the amount of prothrombin present. Final plasma concentrations of 5% or under in the actual clotting mixture are necessary to be able to derive dependable correlations between the rate of clotting and prothrombin activity. 4. For a *full* demonstration of plasma anticephalin (antithromboplastin) activity, clotting mixtures with a high plasma concentration, a moderately strong homologous thromboplastin and inert contacting surfaces (*e.g.* silicone) are essential.

18. Tocantins, L. M., Carroll, R. T., and Holburn, R. R., Trans. Third Josiah Macy, Jr. Foundation Conference on Blood Clotting, 1950, in press.

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Feeding Tests with Some Algin Products. (18580)

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Algin products are widely used in food and pharmaceutical products as stabilizing, thickening, suspending, emulsifying and gel producing agents, because of their pronounced hydrophilic, colloidal properties. They are derivatives of alginic acid which is a polymer of anhydro-β-D-mannuronic acid units extracted from the giant kelp (*Macrocystis pyrifera*). Three algin products manufactured by the Kelco Co. of San Diego, Calif.

covered here are: (a) Dariloid, a sodium alginate standardized to uniform colloidal strength with sugar and dextrin, and treated with phosphate to make it soluble in milk. It is used as a stabilizer in ice cream and other food products. (b) Kelgin, a sodium alginate used in foods as a stabilizer, thickener or gel-forming agent and in pharmaceuticals. (c) Kelcoloid, a propylene glycol alginate used as an emulsifier, bodying agent and stabilizer in foods such as French dressing, and in pharmaceuticals. These algin products are used in aqueous solution at levels of about 0.1 to 0.5% by weight of the finished product.

The feeding studies were conducted under a cooperative agreement with the manufacturer in order that the U. S. Fish & Wildlife Service might obtain data on the nutritive value of seaweed products and be better able to recommend a rational system of utilization of this important natural resource. The products used were purchased on the open market.

Experiments with rats. 10-week comparable feeding tests begun in March, 1939 have been reported for algin (Dariloid) and gelatin(1), and for agar and Irish moss(2). In these experiments the levels of seaweed preparations fed were 5, 10, 20 and 30% by weight of the diet. Similar 10-week feeding experiments were conducted during that time with Kelgin which have not been reported previously. The individual gains in weight, and food and water consumption were quite similar to those of rats fed Dariloid. The mean daily gains of the groups of rats fed the 5, 10, 20 and 30% levels of Kelgin for the 10-week period were 3.81, 3.47, 2.91 and 2.35 g respectively. The mean total food consumed per g gain in weight was 3.20, 3.26, 3.48 and 3.87 g respectively. The mean water consumed per g gain in weight was 5.84, 6.28, 8.83 and 13.74 g respectively. Only 2 instead of 4 out of 6 rats per group survived on the 2 higher levels of Kelgin. The deaths of these 8 rats during the first 2 weeks were apparently not due to Kelgin *per se* but indirectly to the low absorption quality of the basal diet. Long duration experiments were limited for both Dariloid and Kelgin to diets containing the 5% level. Only gross post-mortem studies were done since the animals were permitted to die naturally. Experiments, both short and long duration, with Kelcoloid fed at various levels in the diet were begun in July, 1946. Male albino rats (Yokelson's strain) were used in the experiments with Dariloid and Kelgin. Male and female albino and hooded rats (College Park laboratory strains) were used in the experiments with Kelcoloid. Rats weighing about 50 g and about 4 weeks in age were allotted into groups of about 10. They were individually caged and were offered food and water free-choice. The live weight, and food and water consumption data were collected weekly. A uniform environmental temperature of 80°F was maintained. The diets for rats, except in one instance, consisted of algin at the desired level; casein, 15; lactalbumin, 5; lard, 15; dried brewer's yeast, 5; wheat germ, 2; cod liver oil, 2; salt mixture (U.S.P. XII, No. 2 for vitamin A or D assay) 4, and dextrin and sucrose in about equal quantities to make a total of 100 parts by weight. After the forty-fifth week, 0.5% dried liver extract, Lilly, was added to the diet containing Kelcoloid to determine if additional vitamins might effect a change in texture of feces. No change was noted. A single group of rats was fed the 15% level of Kelcoloid in ground Purina Dog Chow.

The data in Table I indicate no significant effect of the 5% level of algin on longevity. The data in Table II indicate that the group fed the 5% level of algin had similar mean maximum weights and consumed similar quantities of food and water as the controls. The higher levels of Kelcoloid did reduce life expectancy somewhat but not as much as might be expected from the comparatively large intake of food and water. It is surprising how well the rats fed the 15 and 25% levels of Kelcoloid were able to handle the bulky diet which caused loose and smeary feces. The rats fed the 15% level of Kelcoloid in the Purina Dog Chow diet grew very well during

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2. Nilson, H. W. and Schaller, J. W., *Food Res.*, 1941, v6, 641.

TABLE I. Length of Life in Weeks of Rats Fed Different Levels of Algin.

Diet designation	Length of life in weeks											
Control	53	66	83	90	93	95	98	101	104	123	129	132
Dariloid: 5%	30	62	64	67	76	80	89	96	99	128		
Kelgin: 5%	45	67	70	72	83	85	90	93	101	128		
Control	5	14	63	*64*	*73*	75	*76*	*85*	*94*	109		
Kelcoloid: 5%	*10*	*10*	21	37	*41*	*62*	75	76	94	*104*		
15%	1	*1*	*2*	3	*3*	*43*	66	70	*78*	*79*		
15%*	25	36	†									
25%	5	*13*	15	*44*	*57*	*57*	*58*	*66*	79	86		

Italicized data are for female rats.
* These rats were fed the Purina Dog Chow diet.
† Others sacrificed at about 37 wk.

TABLE II. Data on Feeding Tests with Algin Conducted from Weaning to Death of Rats, Except as Noted.

Diet designation	No. of rats	Mean max. wt, g	Mean weekly algin intake, g	Mean weekly basal diet intake, g	Coef. of var. of food intake, %	Mean weekly water intake, ml	Coef. of var. of water intake, %
Control	12	578	0.0	92.2	9	159	19
Dariloid: 5%	10	567	4.5	86.6	14	165	16
Kelgin: 5%	10	577	4.6	88.6	9	168	15
Control	10	292	0.0	61.9	18	130	25
Kelcoloid: 5%	10	292	3.3	63.0	17	149	25
15%*	5	245	10.3	58.5	20	169	21
15%†	10	251	13.9	78.5	14	225	12
25%	10	130	11.3	33.7	37	129	40

* Only rats which lived for at least 3 wk are included.
† These rats were fed the Purina Dog Chow diet, and 8 survivors were sacrificed after 37 wk.

the 37-week period, and the feces were normal in consistency. The early deaths of the rats fed the purified diet containing 20 and 30% Kelgin and 15% Kelcoloid must have been due to inanition which was indicated in the food consumption records. The apparent digestibility of the algin content of Dariloid during the tenth week was 3% for rats fed the 5% level, 36 for the 10, 63 for the 20 and 88% for the 30% level. The amount digested seemed to depend on the concentration in the diet, and may have been dependent on the changed bacterial flora in the intestines. For Kelgin it was none for rats fed the 5% level, 67 for the 10, 76 for the 20 and 78% for the 30% level. For Kelcoloid, 8 out of 23 rats apparently did not digest any algin. The other 15 showed a mean apparent digestibility of 15% irrespective of the level fed. The range in apparent digestibility for individual rats was 0.4 to 34%. The lowest level of algin fed to the rats represents a minimum of 10 and a maximum of 50 times the quantity of algin used in a foodstuff or pharmaceutical product for human use. The rats also had to consume this level in their entire food intake while algin is seldom consumed in more than a single item in the daily diet of humans. The data on longevity, maximum weight, and food and water consumption indicate no adverse trend that can be ascribed to the algin *per se* (Tables I and II). The higher mortality rate, decreased size, etc., associated with the higher levels of algin can be ascribed to the greater bulk, and water absorbing capacity of the colloidal material which seemed to limit the intake of nutrients. There was no differential effect on the two sexes.

In the experiments with Dariloid and Kelgin, the gross necropsy studies showed no recurring symptoms which indicated a toxic condition caused by the algin. In the experiments with Kelcoloid microscopic anatomy studies for all animals were made of the brain, heart, lung, stomach, liver, spleen, kidney, small intestines, large intestines, and ovaries or testes whenever an animal was sacrificed or, if found dead, before decomposition had

advanced too far. As many rats as possible which died during the later part of the experiment were examined. Death of rats fed control and Kelcoloid diets was usually due to myocardial fibrosis, pneumonia and the multiplicity of cumulative processes associated with aging. There were no lesions which could be attributed to a toxemia or to a local irritative intestinal process. Renal calcareous deposits were found frequently in animals that died during the latter part of the experiment in control as well as experimental animals and probably were related to some extraneous dietary constituent. In summary, algin is a wholesome food product as indicated by these feeding tests with rats.

Experiments with mice. Mice weighing 12 to 18 g were allotted into 4 groups which were fed Ground Purina Dog Chow containing 0, 5, 15 and 25% by weight of Kelcoloid, respectively, for one year. The mice were caged individually. None of the mice in the control group died naturally during the year. Mice died during the following weeks in the other groups: 5% level, 13, 48, 48 and 50; 15% level, 13, 13 and 31, and the 25% level, 1, 13, 13, 20 (escaped), 36, 39, 45 and 48. One mouse in the control group, 1 fed the 15% level and 2 fed the 25% level were sacrificed after 39 weeks. The others were sacrificed after a year. The mean maximum weights were 34.5, 34.8, 31.6 and 28.6 g respectively for mice fed the 0, 5, 15 and 25% levels of Kelcoloid. The mean weekly consumption of Kelcoloid, Purina Dog Chow and water was 0 and 31.6 g and 13.1 ml for mice in the control group; 1.7 and 32.5 g and 12.0 ml for mice fed the 5% level; 5.0 and 28.3 g and 13.5 ml for mice fed the 15% level, and 7.9 and 23.8 g and 16.5 ml for mice fed the 25% level of Kelcoloid. There were no outward symptoms of toxicity. No lesions were found during a study of the microscopic anatomy of important organs which could be definitely attributed to any single process. There was, however, a higher mortality rate and smaller mean maximum weight of mice fed the 2 higher levels of Kelcoloid. This apparently was due to the water absorption quality of the diet which caused enough bulk to limit the intake of nutrients.

Experiments with guinea pigs. The pigs were fed Champion Mink Food containing 0, 5, 10 and 15% Kelcoloid respectively. After 2 control pigs and 2 fed the 5% Kelcoloid level died within 8 weeks, the diet was changed to include 50% of an equal mixture of ground yellow corn and standard wheat middlings. In addition 10 ml of tomato juice and a limited quantity of poor grade timothy hay was fed. After 26 weeks the control pigs had final weights of 642, 648 and 708 g and a mean weekly intake of concentrate feed of 155 g per pig. Two of these pigs were replacements after the seventh week. The single pig remaining in the group fed the 5% level of Kelcoloid had a final weight of 627 g and had a mean weekly intake of 146 g of concentrate feed. The pigs fed the 10% level of Kelcoloid had final weights of 557, 585 and 688 g and a mean weekly intake of concentrate feed of 152 g per pig. The pigs fed the 15% level of Kelcoloid had final weights of 570, 602 and 633 g and a mean weekly intake of concentrate feed of 157 g per pig. The study of microscopic anatomy of organs showed no lesions except mild cloudy swellings of the liver and kidneys in any of the control or Kelcoloid pigs. In summary, all pigs grew well after the diet had been altered to include more vitamin C, carbohydrates and roughage. The Kelcoloid was without untoward effect at any level fed in the diet.

Experiments with chicks. Day-old New Hampshire chicks were allotted to 4 groups and fed Purina Starting Mash containing different levels of Kelcoloid. At first the selected levels were 0, 10, 25 and 50% Kelcoloid. During the first day it became apparent that the chicks receiving the 25 and 50% levels could not eat the diet. It oftentimes formed large masses on the beaks. The chicks fed these levels for 1 day were then fed the control diet for 1 week. At the close of this period they were fed diets containing 5 and 15% Kelcoloid. The data in Table III show that the Kelcoloid even in the lowest level apparently reduced the growth rate although these chicks ate about the same as the control chicks. The Kelcoloid may have inter-

TABLE III. Data on Feeding Studies with Chicks.

Diet designation	No. of weeks	No. of chicks		Mean wt of survivors, g	Mean weekly intake	
		Initial	Close		Kelcoloid, g	Basal diet, g
Control	7	13	11	602	0	189
Kelcoloid: 5%	7	13	12	505	10	195
10	7	12	6	316	15	130
15	3	13	4*			

* Survivors were killed after 3 wk for study of microscopic anatomy.

TABLE IV. Data on Feeding Tests with Cats.

Identification	Duration, days	Weight		Mean daily intake		
		Initial, g	Final, g	Kelcoloid, g	Pard, g	Salmon, g
LMR	14	2335	2144	6.4	40.7	32.3
LR	14	2475	1973	15.8	68.3	37.9
1*	88	2030	975	7.4	66.2	22.1
2	110	2077	2007	5.0	91.2	27.8
3	57	2273	2125	0.0	95.6	33.4
	54	2125	1735	8.9	80.4	26.8
4†	45	2031	1152	5.4	89.0	32.4
5	90	2192	2197	0.0	99.4	29.8
6‡	33	1960	1250	14.6	72.4	31.6
8	90	2050	1004	6.0	54.2	16.3
Mother cat		2421	2742			
Kitten		542	1485			

* This cat did not eat well towards latter part of period. It seemed strong in the evening, but died during the night.

† This cat seemed suffering from a bad cold for 2 days before it died. The eyes were swabbed with boric acid solution, and 20,000 units of penicillin were given intraperitoneally.

‡ This cat was extremely emaciated and practically moribund, so it was sacrificed.

fered with the utilization of nutrients. In the 2 higher levels there was evidence that the physical condition of the diet limited food intake sufficiently so that the chicks could not survive or grew very poorly. It was almost a physical impossibility for the chicks to eat the diet containing the 15% level of Kelcoloid. A variety of lesions of minor importance was seen in the organs at irregular and insignificant intervals. Although the chicks had not survived or grown well in many instances, it was concluded from this study that the anatomical lesions indicated slight evidence of transient reversible tissue changes in both control and Kelcoloid chicks. There was no evidence of chronic toxicity.

Experiments with cats. The cats were fed a diet of canned Pard dog food and canned salmon for at least a month until there were no gross symptoms of disease or parasites. One cat had a litter of kittens of which 1 survived. The mother cat and kitten were fed the same diet and were used as a negative control at the close of the experiment. Two cats were sacrificed after a 2-week feeding test. The remainder of those that survived were fed from 88 to 111 days and were sacrificed. The quantity of diet offered to all cats except the mother cat and kitten was limited to 100 g of Pard diet containing 0, 5, 10 or 15% Kelcoloid, and 30 g of canned salmon. None of the cats fed Kelcoloid would consume any more than this quantity of food. Cat 3, during the control period, and cat 5 barely maintained their weight on this quantity of food. It is not surprising that the other cats lost weight (Table IV). The cats had trouble eating the diet containing Kelcoloid seemingly because of the physical texture. They had difficulty in swallowing, also. The cats which received the higher levels of Kelcoloid passed 2 or 3 stools daily which were soft in texture but were fairly well formed. There did not seem to be any indications of a chronic toxicity. The eyes of the cats were clear, and the cats kept themselves neat and clean. Inspection of the organs after necropsy, both macro- and microscopic, of both control and

Kelcoloid fed cats indicated that the lesions which were found were of no significance insofar as any specific disease process was concerned. Kelcoloid appeared to be a wholesome food.

Discussion. The algins are partly digestible, but it is not known whether the animal directly or bacteria in the intestinal tract utilize these products. This is of no great nutritional significance since the products are used in only very small quantities in foods.

The difficulties encountered with diets containing high levels of algin may be attributed to the high water absorbing and bulking capacities of this high viscosity product. At the levels fed, water would produce a sticky, heavy paste or semi-gel causing difficulty in chewing and swallowing or a reduction or normal nutrient intake. This kind of physical effect may be expected with any product of this type. Nilson and Schaller(2) reported similar conditions when Irish moss was fed to rats.

In commercial usage the products are, of course, fully hydrated and used at levels of 0.1 to 0.5%. Thus the lowest level fed in these experiments, namely 5%, represents 10 to 50 times the amount that would be consumed in the daily diet of humans if everything eaten contained algin.

Conclusions. The data indicate that these algin products are free from any deleterious substances which adversely affect health when fed at levels which are at least 10 times higher than when used in foods or pharmaceutical products. The difficulties experienced with feeding the higher levels seem to be due, at least in greater part, to the physical texture imparted to the diets fed. This should not be a problem in the commercial use of the product. The data indicate that the three algin products which were tested are wholesome.

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Reversible Agglutination of Trypsin Treated Erythrocytes by Normal Human Sera.* (18581)

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(Introduced by Mario Stefanini)

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Human erythrocytes, when treated in various ways, are susceptible to agglutination by apparently normal, compatible human sera. This effect is entirely independent of the usual blood groups and their agglutinins. Thomsen (1) first demonstrated that red cells exposed to certain bacteria can be agglutinated by most human sera. Friedenreich(2) extended these observations by showing that an enzyme derived from the bacteria was responsible for the change in the properties of the red cell. Since then it has been shown that exposure of red cells to Cholera Vibrio filtrates(3), viruses of the mumps-influenza group(3), and periodate ions(4) renders these cells panagglutinable by human sera. That the agglutinins in human sera responsible for these effects are varied and distinct has been shown by Stewart(5) and Moskowitz and Treffers (6). Brief mention is made by Wheeler *et al.*

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