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SOME NUTRITIVE PROPERTIES OF CORN

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SUMMARY

1. Corn alone is an adequate diet for adult pigeons for maintenance, at least during a period of 1 year.

2. Corn bran contains relatively large amounts of antineuritic substances, or substances similar to those contained in rice polishing, called vitamines by Funk, and water-soluble B by McCollum.

3. Five grains of corn a day will supply the requirement of an adult pigeon for this substance or substances.

4. Corn germ contains some of the food accessories called fat-soluble A by McCollum, but very little of the water-soluble B.

5. A fairly rapid but abnormal growth can be induced in young chickens by force feeding them on a diet of corn and salt mixture.

6. Some of the benefit derived by adding crude casein to a diet of corn and salt mixture is due to the fat-soluble A which it contains. This may be removed by thoroughly extracting, or destroying by autoclaving at 45 pounds pressure for 2 hours.

7. Autoclaving the casein does not destroy its efficiency as a protein for producing growth in young rats.

The author gratefully acknowledges his indebtedness to Vice-president J. T. Willard, who kindly granted the funds necessary for conducting these experiments, and to Dr. A. G. Hogan, who coöperated in the work with autoclaved casein. The author further acknowledges the assistance of both in planning and conducting the work.

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SOME NUTRITIVE PROPERTIES OF CORN[1]

JOSIAH SIMPSON HUGHES

Many practical feeders have for a long time considered corn alone an inferior ration for growing animals. Some even claim that a feed consisting largely of corn is poisonous to some animals. Among the first experiments to show the inadequacy of corn were those of Sanborn (1884) who states: "Experience convinces me that the exclusive use of corn for a feeding ration is detrimental to a vigorous, healthy, muscular development, producing a pig easily subject to disease."

Henry (1889), who obtained similar results, found that the breaking strength of the bones could be increased by the addition to the corn ration of either wood ashes or bone meal.

Forbes and Keith (1914) as a result of their experiments on the specific effect of rations on the development of swine conclude that, "Corn, which is characterized by low protein, calcium, and phosphorus content, in comparison with the better balanced rations, produces under-size, over-fat animals, with small viscera and deficient muscular development, and bones which lack in size, strength, and ash per unit volume."

In experiments conducted at the Kansas State Agricultural Experiment Station during the past 6 years, Waters and his co-workers found that both ash and protein must be added to corn to obtain an adequate ration for pigs.

Forbes (1914) attributes the mineral deficiency of corn to its low calcium, magnesium and phosphorus content. Hogan (1917) found the addition to corn of potassium phosphate had very little, if any, beneficial effect, while calcium lactate is almost as efficient an addendum for corn as the complete ash mixture[2] from which he concludes that calcium is the most important mineral deficiency of corn.

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1. This bulletin was presented by the author as a thesis in the Graduate School of the Ohio State University in partial fulfillment of the requirements for the degree of doctor of philosophy, June, 1917.

2.

	Grams
Ca lactate	468.0
K_2HPO_4	280.8
Nacl	123.1
Na citrate	31.2
Fe citrate	23.8
$MgSO_4$	31.7

The protein deficiency of corn seems to be due to the poor quality rather than to the quantity of protein it contains. For corn, which usually contains about 9 percent protein, will produce a very slight growth, even when all the other necessary dietary factors are present in sufficient amounts, while a diet containing this amount of protein in the form of milk albumen has been shown by Osborne and Mendel (1915) to produce a maximum growth. This deficiency is undoubtedly due to the high percent of zein, a protein which contains neither lysine nor tryptophane, and which is unable to support life. The other proteins of corn are able to produce growth as shown by the experiment, described later, in which chickens were found to grow when they were forced to consume large amounts of corn. The same conclusion is drawn from the experiments of Osborne and Mendel (1914), Hart and McCollum (1914) and Hogan (1917), in which they were able to produce rapid growth by adding corn proteins other than zein to a corn diet. Hogan (1917) has shown tryptophane to be the first limiting factor in the protein deficiency of corn and lysine the second.

Dietary experiments during recent years have shown that a diet, to promote the physiological well-being of an animal, must contain, in addition to the proper proteins, carbohydrates, fats, minerals, and small amounts of other unidentified substances. Hopkins (1912) suggested that the benefit derived from the addition of small amounts of milk to a diet was due, in part, to some unknown substances it contained. Cooper and Funk (1911) showed that additions of rice polishing to a diet would prevent polyneuritis or beri-beri. The first conclusive evidence of the necessity of small amounts of unknown substances in the food was obtained by Funk (1911). He isolated a crystalline substance from rice polishings and from yeast, which, in amounts as small as a fraction of a milligram, was effective in curing polyneuritis in pigeons. He called this substance vitamine, since it was found to contain about 10 or 12 percent of nitrogen. Stepp (1912), McCollum (1913) and Osborne and Mendel (1913) found that satisfactory growth cannot be obtained with a restricted diet of purified food substances, but that normal growth results if both butter fat and "protein-free milk" are include in the diet. They attributed the beneficial property of the butter fat and the "protein-free milk" to some unknown compounds which they contain. Mc-

Collum (1915) has shown that these accessory food substances can be divided into two classes on the basis of their solubility, one which is soluble in fat and fat solvents and another which is soluble in water and alcohol. He has designated the former as fat-soluble A and the latter as water-soluble B. The crystalline material which Funk isolated from rice polishings and yeast belongs to the water-soluble B class. This class of substances possesses antineuritic properties, while the fat-soluble A class does not seem to possess these properties as they are not effective in preventing or curing polyneuritis.

Williams (1916) prepared synthetically a form of a hydroxy-pyridine, adamine, betaine, and nicotinic acid which possess antineuritic properties. He concludes, "that the curative form of a hydroxy-pyridine is a pseudo-betaine and that a feature conforming more or less closely in structure or energy conditions to the betaine ring is probably an essential characteristic of antineuritic vitamines."

The work of Funk (1911) suggested that the deficiency of corn might be due in part to an insufficient amount of vitamines. So, in the fall of 1912, at the suggestion of Dr. J. F. Lyman, the writer began a study of the vitamine content of corn and its relation to the inadequacies of corn to produce a normal growth. Since this time Funk (1913), McCollum, Simonds and Pitz (1916-17), and Hogan (1916) have published results of experiments bearing on this question. For the most part the results of our experiments are in accord with those already published.

EXPERIMENTS WITH PIGEONS

ACCESSORIES IN CORN. — In some preliminary experiments corn was shown to contain relatively large quantities of antineuritic substances, similar to those found in rice polishings, by curing pigeons with it in which polyneuritis had been induced by feeding polished rice. As few as 5 grains of corn were found to be sufficient to cure a pigeon in from 3 to 10 hours, even when the pigeon had become so paralyzed that it could not swallow and the corn had to be forced into the crop. In one case 7 grains of corn were administered and after 10 hours, when recovery was apparently complete, autopsy showed that 5 grains were yet in the crop. The cure had been effected by the 2 grains which had been digested together with any material-which could have been dissolved from the 5 grains remaining in the crop.

A number of pigeons were cured with an alcoholic extract of corn bran which was prepared by extracting the bran with 95 percent alcohol and distilling off the alcohol under reduced pressure. The residue was then extracted with water and a portion of the water extract representing 50 grams of bran were given to the pigeon.

Corn was also shown to contain food accessories by the fact that a pair of pigeons remained normal for a year and gained slightly in weight on a diet of corn alone.

These results are in harmony with Funk's (1913) statement that corn bran contains vitamines.

DISTRIBUTION OF ACCESSORIES IN THE CORN GRAIN. — In order to study the distribution of the accessories in corn, five pens of pigeons were fed with as many different diets. Pen 1 was given polished rice; Pen 2, corn germs obtained from the Corn Products Refining Company; Pen 3, pearl hominy; Pen 4, 25 percent corn bran and 75 percent polished rice; and Pen 5 was given commercial corn meal.

The results for Pen 1 which was fed polished rice are given in Table I.

TABLE I.—*Loss of weight of pigeons on a diet of polished rice*

Day of experiment	Weight of pigeons in grams			
	No. 9	No. 10	No. 15	No. 16
1	393	299	307	310
4	390	304	317	279
8	376	290	312	255
12	355	275	302	232
16	315	224	254	226
20	296	192*	220	221
24	250	....	206	195*
28	237*	....	200	....

* Severe polyneuritis

The pigeons in this pen lost weight very rapidly, the average loss being 121 grams.

The average length of time before the onset of polyneuritis was 25 days.

The pigeons in Pen 2 which were fed corn germs developed polyneuritis in an average of 28 days, which is just 3 days longer than for the polished rice. The average loss of weight was only 82 grams, or 39 grams less than the average loss on polished rice. The detailed results are given in Table II.

TABLE II.—*Loss of weight of pigeons on a diet of corn germs*

Day of experiment	Weight in grams			
	3	4	19	20
1	327	318	325	397
4	332	315	323	385
8	323	297	300	372
12	325	289	301	370
16	314	287	295	362
20	305	272	299	337
24	275	240*	293	292*
28	260*	....	284	....
32	....	....	288	....
36	....	....	247*	....

* Severe polyneuritis

These results would seem to show that the corn germ is very little more efficient in preventing polyneuritis than polished rice, but that it is much more efficient in preventing loss of weight.

A pigeon receiving a ration consisting of 75 percent corn germ and 25 percent corn bran remained normal for 50 days, with a slight gain in weight. The antineuritic substance in this case was supplied by the corn bran.

Table III shows the effect of feeding pearl hominy, i. e., corn with the germ and bran removed.

TABLE III.—*Loss of weight of pigeons on a diet of pearl hominy*

Day of experiment	Weight in grams	
	No. 7	No. 8
1	318	305
4	320	310
8	317	306
12	311	291
16	304	265
20	291	252
24	242	214
30	210*	214*

* Severe polyneuritis

All pigeons lost weight very rapidly, the average loss being 99 grams in 30 days.

Pigeon No. 7 died on the thirty-first day of the experiment and No. 8 was in the advanced stages of polyneuritis. The latter was then put on a diet consisting of 80 percent pearl hominy, 10 percent corn bran and 10 percent corn germ. In 20 days it had reached a weight of 321 grams, or 17 grams more than the weight at the beginning of the experiment. It remained normal on this diet for 30 days when the experiment was discontinued. These results' show pearl hominy to be similar to polished rice in being deficient in food accessories.

The pigeons in Pen 4 receiving polished rice and corn bran did not develop polyneuritis, but gradually lost weight until one had lost 198 grams when it died on the 139th day of the experiment. This was a loss of 51 percent of its original weight. The eyes of the pigeon in this pen became affected 4 or 5 days before death, and watered profusely. These results seem to show that 25 percent of corn bran furnished sufficient amounts of antineuritic substances, but that there were some other essential substances lacking. Corn germ was added to the diet of one pigeon from this pen and it regained its lost weight and remained normal until the experiment was discontinued. This shows that the corn germ contains some essential substance in relatively larger amounts than the bran. This is perhaps the accessory which McCollum (1915) has called fat-soluble A.

The pigeons in Pen 5 receiving commercial corn meal remained normal for 100 days, at which time the experiment was discontinued. This meal was made by a local firm by grinding the corn and bolting out the bran. If it had been made by the process in use in some larger mills, which consists of first removing the bran coat and germ and then grinding, the results would perhaps have been more like those obtained with pearl hominy. Funk (1913) suggested that pellagra is due to a diet consisting largely of cornmeal which has had all the bran removed.

These experiments show (1) that the antineuritic substances of corn are found in the bran; and (2) that there is some other essential substance or substances contained in the germ.

THE AMOUNT OF CORN NECESSARY TO PREVENT POLYNEURITIS IN PIGEONS.—In order to determine the amount of corn necessary to prevent the onset of polyneuritis, a number of pigeons were fed polished rice *ad libitum* and in addition were given different amounts of corn. The results of this experiment are shown in Table IV.

TABLE IV.—*Loss of weight of pigeons receiving polished rice ad libitum and from 1 to 10 grains of corn per day*

Days	1 grain per day		3 grains per day		5 grains per day		10 grains per day	
	No. 26	No. 27	No. 28	No. 29			No. 112	No. 113
1	309	323	300	315	292	315	280	302
4	298	240	278	302	285	319	284	315
8	286	227	265	290	280	316	278	312
12	264	227	249	289	282	308	274	303
16	231	214	225	270	275	301	264	305
20	215	189	235	263	270	293	270	307
24	200	195	230	240	276	295	272	311
28	190	190	228	235	263	287	270	295
32	179	175*	201	238	263	286	272	244
36	166*	....	199	212	254	279	270	301
40	....	....	210	202	260	270	275	307
44	....	....	190	205	257	271	278	312
48	....	....	210	202	251	275	280	311
50	....	....	198	203	245	278	275	307

*Died from polyneuritis

The pigeons receiving 1 grain of corn a day developed polyneuritis in 36 days, on the average, which is 10 days longer than the average for polished rice alone. The average loss of weight was 147 grams.

The pigeons receiving 3 grains of corn a day lost weight rapidly, but did not develop polyneuritis, while those receiving 5 grains a day lost weight very slowly. Those receiving 10 grains a day remained normal throughout the experiment.

Since it required only 5 grains of corn to effect complete recovery in the case of a pigeon in the advanced stages of polyneuritis, it was rather surprising that 1 grain a day would not prevent its onset. The fact that those receiving 3 grains a day did not develop polyneuritis, but did lose weight rapidly may be explained on the assumption that the antineuritic substance is found in corn in relatively larger amounts than some other essential substances.

This is in accord with McCollum's (1916-17) recent view, that corn contains abundance of water-soluble B, but relatively smaller amounts of the fat-soluble A.

EXPERIMENTS WITH SQUIRRELS

The corn germs used in the experiments with pigeons had been subjected to a slight extraction since in the process of their separation the corn was soaked before it was crushed. A sample of corn germ was obtained from the pearl hominy factory, but as it contained considerable bran, it could not be used to study the antineuritic property of the germ. Since squirrels will cut the germ from corn and discard the rest of the grain, they were chosen for an experiment. Rats were tried, but they ate a portion of the grain besides the germ.

Two young fox squirrels were obtained, one weighing 185 grams and the other 190 grams. One was fed ground corn so it would be compelled to eat the entire grain. The other was given the same kind of corn, but it was unground, thus allowing the squirrel to cut out the germs. The one eating the corn germs alone began to show signs of paralysis, particularly in the forelegs, on the thirtieth day of the experiment. This condition continued to grow worse until the forty-seventh day, when the squirrel died. The one eating the entire grain remained normal for 50 days, but did not increase in weight. It was then given the unground corn. After it had been eating the corn germs for 23 days, it became paralyzed in the forelegs, as had the other.

While one cannot draw definite conclusions from an experiment involving but two animals, these results in connection with the experiments on pigeons indicate that the corn germ is lacking in some substance or substances which are necessary to the maintenance of animals. This perhaps is the antineuritic substance which Funk has termed vitamines and McCollum water-soluble B.

EXPERIMENTS WITH CHICKENS

CORN CONTAINS ACCESSORIES.—In some experiments with milo maize it was found that young chickens receiving this grain alone developed polyneuritis and that a few grains of corn were sufficient to cause a marked improvement. A typical illustration of these results is shown in Fig. 1. No. 1 shows a chicken which had received nothing but milo maize for 26 days. At the end of this time it was unable to stand and showed other symptoms of advanced stages of polyneuritis, No. 2 shows the same chicken 24 hours after 10 grains of corn had been forced into its crop. These results, together with those previously presented, show that corn contains some antineuritic substances, which are lacking in milo as well as in polished rice.

FORCE FEEDING. — It has been noted by many investigators (Osborne and Mendel 1915) that an animal, when limited to a diet which is deficient in some essential constituent, will not

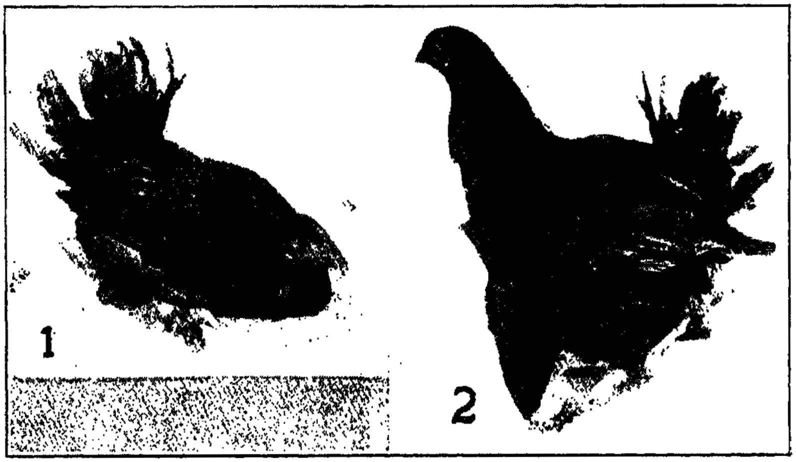


FIG. 1.—No. 1, a young chicken fed exclusively on milo maize. No. 2, the same chicken 24 hours after 10 grains of corn had been forced into its crop

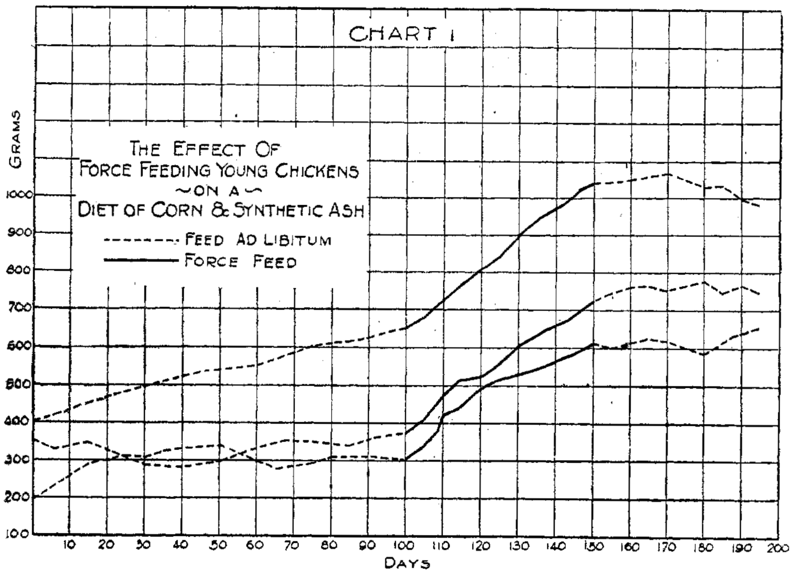
consume enough food to provide the required amount of this constituent. Waters, for example, found that swine on a diet of corn and salt mixture would consume much larger amounts if a small amount of protein was added than without this addendum, but no means were found to induce them to consume more corn without adding protein to it.

This is not true with chickens, however, for they can be forced to eat, the only limit being the capacity of their crop and the ability of the digestive organs to digest the food. This force feeding is very often employed in commercial fattening plants. With these facts in mind, it was decided to force feed a number of young chickens on a diet of corn and a synthetic salt mixture, in order to determine whether or not the increased amount of food would provide a sufficient amount of all the dietary factors to induce a more rapid growth.

For this purpose four White Leghorn chickens weighing about 300 grams each were selected. They were fed *ad libitum* on a diet of corn and salt[1] mixture for 100 days in order to determine the average amount of feed consumed per day and the

1.	$Ca_3(Po_4)_2$	10 parts
	K_2HPo_4	37 "
	Nacl	20 "
	Na citrate	15 "
	Ca lactate	8 "
	Fe citrate	2 "
	$CaCo_3$	92 "

average gain. It was found, as shown in Chart 1, that with an average consumption of 18 grams per day they made an average gain of 121 grams in the 100 days, or about 1.2 grams per day per fowl. At the end of this period, they were forced to consume 60 grams a day, or three and one-third times as muck as they had been eating. During 2 months they made an average gain of 381 grams, or 6.3 grams per day per fowl. Thus



the increase in the amount of feed consumed produced an increase in the rate of growth, which seems to show that the proteins of corn will produce a fairly rapid growth if consumed in large enough amounts.

These results agree with those of Hart and McCollum (1914) who showed that a fairly rapid growth may be induced in swine by using a diet of corn, corn proteins and salt mixture. They also agree with those of Osborne and Mendel (1914) and Hogan (1916) who showed that rats will make almost a normal growth on a diet in which all the protein was obtained from corn.

The chickens in our own experiments, however, did not develop normally, as can be seen in Fig. 2. The ruffled condition of the feathers, the crooked toes and the lack of development of secondary sexual characters are noticeable. They walked with a peculiar halting gait.

The ruffled feathers seem to be a condition similar to the rough coat of fur obtained on rats and other animals suffering from malnutrition, and as in the case with the rough coat of fur, is often accompanied by an eczematous condition of the skin.

Osborne and Mendel (1916) attributed the ruffled feathers in their experimental chickens to the large amount of handling necessary in feeding and weighing. We have found, however, in experiments with about 200 young chickens, that the feathers do not show this ruffled condition if the chickens are receiving an adequate diet, but that in most of the cases when the diet was inadequate the feathers were ruffled. This abnormal condition can usually be found in any large flock of young chickens where proper care in feeding is not taken.

FIG. 2.—White Leghorn cockerel 8 months old, typical of the chickens force fed on a diet of corn and salt mixture

The lack of development of the secondary sexual characters was very noticeable. Even when the chickens were 6 months old, and should have been almost fully developed, it was difficult to distinguish the sex.

These abnormal conditions show that a diet consisting of corn and synthetic salt mixture is not adequate for normal growth, although when consumed in large enough amounts it will produce fairly rapid growth.

CORN SUPPLEMENTED BY EXTRACTED CASEIN.—The above experiment did not show whether the inadequacy of corn to produce normal growth when supplemented with ash is due to an insufficient amount of food accessories or an inadequacy of the protein content. One should be able to answer this question by studying the adequacy of a diet of corn and salt mixture supplemented by an adequate protein which is free from accessories.

To obtain data on this point, young chickens were fed a diet of corn and salt mixture supplemented with casein in which the accessories were removed as much as possible. To free the casein from food accessories, some was extracted and some was autoclaved as described in the following experiments.

In the first of these experiments the casein prepared from centrifuged milk was extracted four times with 95 percent alcohol, but as this was found to remove only a part of the soluble substances, a second experiment was conducted in which the casein was extracted four times with 95 percent alcohol and then four times with petroleum ether. After the ether had been removed by heating in a vacuum oven at 50° Centigrade the casein was placed in a large percolator through which very dilute acetic acid was allowed to percolate for 5 days. It was then dried with alcohol at room temperature.

The alcoholic extract when distilled under reduced pressure at about 500° Centigrade gave a residue which was very effective in curing pigeons of polyneuritis induced by feeding polished rice. The residue obtained by the distillation of the petroleum ether extract showed none of the curative properties possessed by the residue from the alcoholic extract. In these experiments, enough of the casein was added to make 4 percent nitrogen, in the diet, which also contained 5 percent of the salt mixture used in the force feeding experiment.

The result of the first experiment in which the casein was extracted three times with alcohol is shown in Table V.

TABLE V.—*Showing rate of growth of young chickens on indicated diet*

Days	Weight of chickens in grams								
	Corn, salt			Corn, salt, extracted casein			Corn, salt, casein		
	No. 15	No. 17	No. 16	No. 10	No. 19	No. 11	No. 8	No. 2	No. 16
1	220	330	460	265	345	435	240	340	450
7	240	365	505	348	405	510	295	433	557
14	260	420	555	425	415	585	400	545	698
21	255	410	545	483	540	652	485	593	815
28	220	480	550	565	625	720	580	730	868
35	245	486	510	680	690	865	635	815	860
42	270	540	535	730	830	912	700	880	990
49	280	555	575	730	858	820	705	965	1160
56	305	555	586	820	850	830	750	1030	1290
63	310	Died	620	820	895	825	770	1080	1345
70	....	....	....	790	915	840	....	....	....

The chickens fed the extracted casein grew much more rapidly than those fed on corn alone, but not quite so rapidly as on corn supplemented with the same percent of unextracted casein.

The results of the second experiment in which the casein was extracted with alcohol and then with ether, are shown in Table VI and Chart 2.

TABLE VI.—*Growth of chickens on a diet of corn, extracted casein and salt mixture*

Weeks	Weight of chickens in grams								
	Extracted casein			Unextracted casein			Corn, and salt		
	No. 19	No. 16	No. 10	No. A 10	No. A 8	No. 13	No. 25	No. 6	No. 20
0	165	165	165	185	180	151	260	215	305
1	200	185	185	252	250	192	277	215	305
2	251	215	207	275	307	218	300	247	327
3	214	224	193	286	388	240	330	230	270
4	214	320	204	355	455	272	330	240	245
5	165	390	300	440	590	340	360	265	Died
6	Died	495	315	525	115	400	375	275	....
7	....	560	400	690	830	495	390	300	....
8	....	630	400	755	910	585	440	360	....
9	....	675	360	830	995	680	445	375	....
10	....	675	Died	940	1055	785	440	375	....
11	....	710	....	1080	1160	880	....	400	....
12	....	710	....	1110	....	940	....	....	....
13	....	690	....	Experim	ent disco	ntinued	Experim	ent disco	ntinued
14	....	805*	....	....	....	....	....	....	....
15	....	940	....	....	....	....	....	....	....
16	....	1065	....	....	....	....	....	....	....
17	....	1205	....	....	....	....	....	....	....
18	....	1315	....	....	....	....	....	....	....

* 10 percent butter added

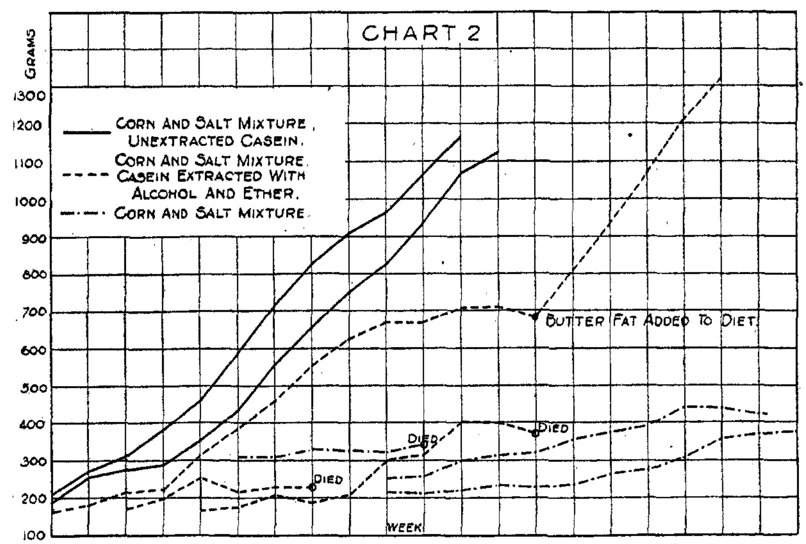
In this experiment, two of the chickens receiving the diet containing the extracted casein died and the other gained only 425 grams during 14 weeks. At the end of this period, 10 percent of butter fat was added to the diet, which resulted in a very marked increase in the rate of growth.

These results show that thoroughly extracted casein is not nearly as beneficial an addendum as the unextracted. Evidently some of the substances extracted with alcohol and petroleum ether are essential to growth and are not present in corn in adequate amounts. These results are in accord with McCollum's findings (1916-17) that corn does not contain enough fat-soluble A to produce normal growth. These results show further that these substances are contained in butter fat.

CORN SUPPLEMENTED BY AUTOCLAVED CASEIN.—In these experiments the casein, instead of being extracted as in the previous experiment, was autoclaved in order to destroy the beneficial properties of the food accessories which it contained. While there has been much conflicting data in regard to the stability of the accessories toward heat, most of the evidence seems to show that the water-soluble substances are very susceptible, while the fat-soluble ones are not destroyed except by prolonged heating at a higher temperature.

Osborne and Mendel (1915) found that live steam could be passed through butter fat for 2½ hours without destroying the accessories which it contains.

McCollum (1915) obtained similar results for the accessories and found also that when milk is heated to boiling it loses some of its nutritive efficiency. He attributes this loss



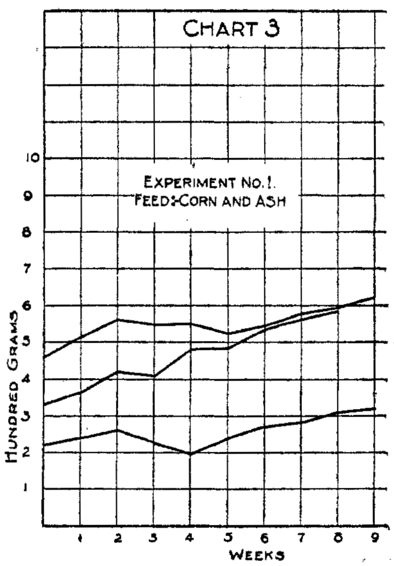
to "changes wrought in the casein." This conclusion, however, is not in accord with the results of the following experiments.

The casein used in these experiments was autoclaved for 2 hours each at 15, 30 and 45 pounds pressure. That some hydrolysis of the casein takes place under these conditions is shown by the fact that 12 percent of the nitrogen of the casein autoclaved at 15 pounds pressure is soluble in water, while 22.8 percent of that autoclaved at 30 pounds pressure, and 30.7 percent of that autoclaved at 45 pounds pressure is soluble. Also a substance is produced by autoclaving which is insoluble in dilute alkali.

When the casein was autoclaved at 15 pounds pressure, 28.4

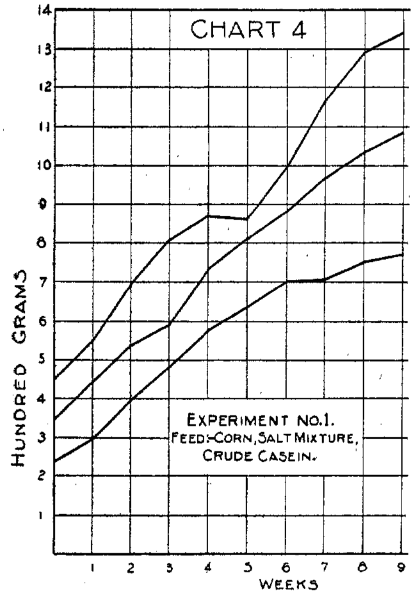
percent of the nitrogen was insoluble in dilute alkali, while only 3.3 percent was insoluble in alkali when autoclaved at 30 pounds pressure, and 1.3 percent when autoclaved at 45 pounds. Of the casein autoclaved at 15 pounds there was 65.5 percent of the phosphorus soluble in water, 69 percent of that autoclaved at 30 pounds was soluble, and of that autoclaved at 45 pounds pressure, 71.8 percent was soluble. This shows that a much higher percentage of the phosphorus than of the nitrogen is converted into a water-soluble form by autoclaving. In the case of the nitrogen, the extent of the hydrolysis seems to depend upon the temperature at which it is autoclaved, while in the case of the phosphorus there is almost as much water-soluble when autoclaved at 15 pounds as when autoclaved at 45 pounds.

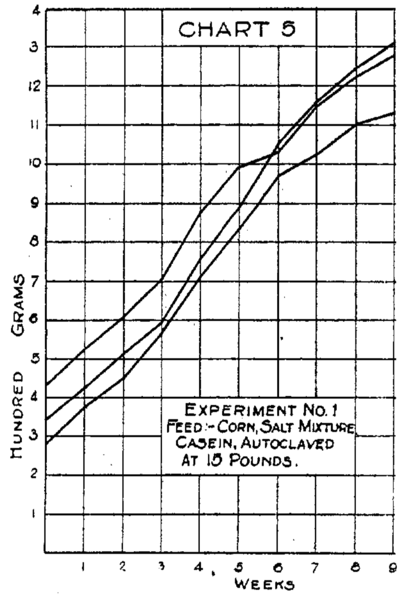
Of the sample autoclaved at 15 pounds, 54.9 percent of the phosphorus was changed to the inorganic form, at 30 pounds 57.4 percent was changed to the inorganic form, and at 45 pounds 57.7 percent.

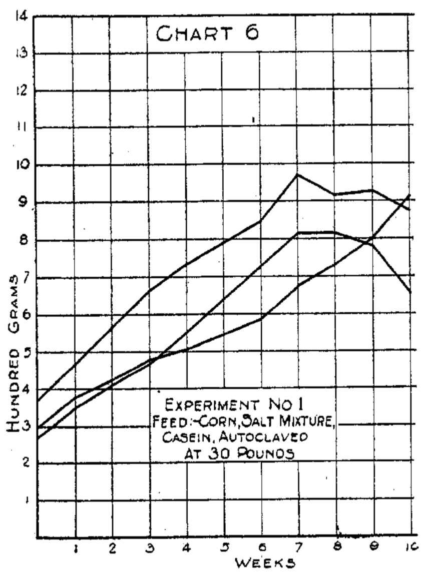


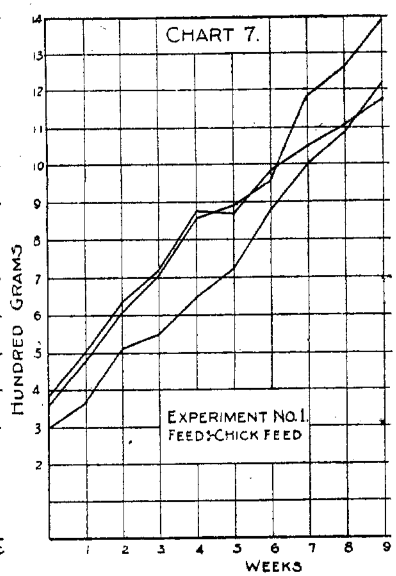
In the experiments, enough of the casein was added to make 4 percent nitrogen in the diet, which also contained 5 percent of the salt mixture used in the force feeding experiment. Both the corn and the casein were ground fine enough to prevent any selection in eating. The chickens were fed all they would consume in the morning and afternoon. In most cases the feed was moistened with water at the time of feeding. The chickens were kept in a well lighted and ventilated room. The board floor of the pens was covered with dean coarse sawdust which was changed frequently. The windows and doors were screened so there were no flies or other insects for the chickens to eat.

EXPERIMENT I.—Three experiments were conducted. In the first of these were five pens of three Plymouth Rock chickens each. Pen 1 was fed corn and salt mixture; Pen 2, corn and salt mixture supplemented by crude casein; Pen 3, corn and salt mixture supplemented with casein autoclaved at 15 pounds; and Pen 4, corn and salt mixture supplemented with



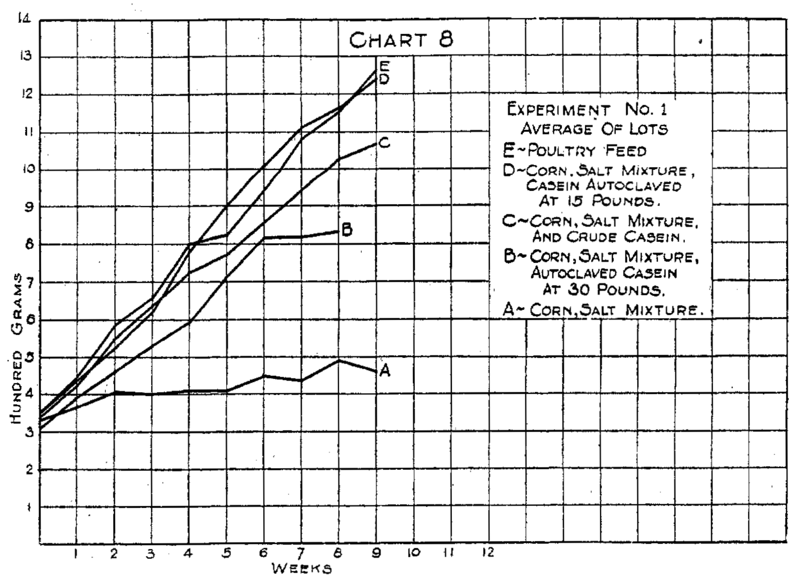






casein autoclaved at 30 pounds. Pen 5 was fed on a chick feed used at the College Poultry Farm, which consisted of corn meal, bran, meat meal, charcoal and oyster shell.

The rate of growth of these chickens is shown in Charts 3, 4, 5, 6 and 7. Chart 8 shows the average rate of growth of each of the five pens. It will be noticed that the most rapid growth aside from Pen 5, which was fed chick feed, was made by those chickens receiving casein autoclaved at 15 pounds. Those receiving casein autoclaved at 30 pounds made a fairly rapid gain at the beginning, but ceased to grow after 6 weeks.



These results can be explained by assuming that autoclaving casein at 15 pounds for 2 hours does not reduce the efficiency as a supplement for corn, but that autoclaving at 30 pounds for 2 hours destroys its efficiency to some extent. So far as this result is concerned, the loss of efficiency might be due either to the alteration which takes place in the casein or to the destruction of the accessories from the milk which were not separated from it in the process of preparation. As experiments with rats, to be discussed later, show that autoclaving at this temperature and time does not destroy the nutritive efficiency of the casein itself to any great extent, the

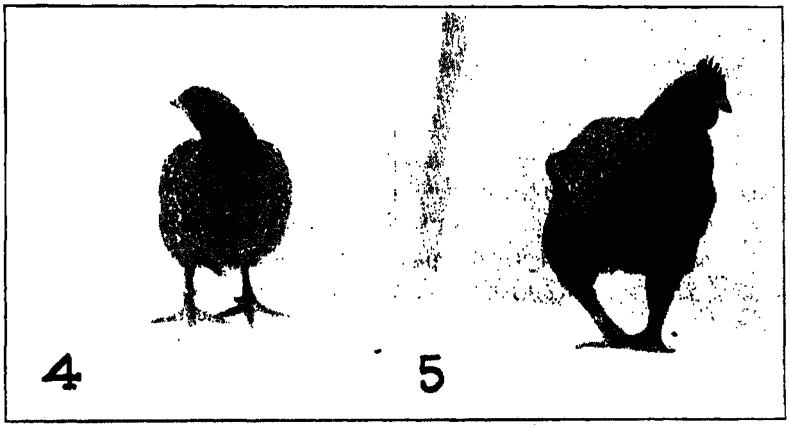


FIG. 3.—No. 4 is a chicken from Pen 2, Experiment 1, which received a ration of corn, casein, and salt mixture. No. 5 is a chicken from Pen 3 which received a ration of corn, salt mixture, and casein, autoclaved at 15 pounds pressure for 2 hours

loss of efficiency must be due to destruction of accessories. This would be in accord with the previous experiment in which it was found that thoroughly extracted casein was not an efficient addendum for corn.

It will be observed (Fig. 3) that the secondary sexual characters, especially the comb, are developed to a much greater degree in the birds from Pen 3 which were fed on a diet containing casein autoclaved at 15 pounds than in the normal birds from Pen 2 which were fed crude casein in addition to the corn and salt mixture. The edematous condition of the legs is also especially noticeable. The abnormal development of the bones of the legs is shown in Fig. 7.

EXPERIMENT II.—It was thought that the abnormal development of the leg bones might be due to lack of exercise rather than to the feed, since in the experiment just described the pens were only 4 feet square in each of which three chickens were kept. Experiment No. 2 was planned to determine this point. In this experiment there were two pairs of pens. One of each pair was 7 feet square and the other 4 feet long and 2 feet wide. Pens 1 and 2 received corn and salt mixture and casein. Pens 3 and 4 received corn and salt mixture and casein autoclaved at 30 pounds. Pen 5 was 7 feet square and the chickens in it received corn and salt mixture. The rate of growth of these chickens is shown in Charts 9 to 13, and Figs. 4 and 5.

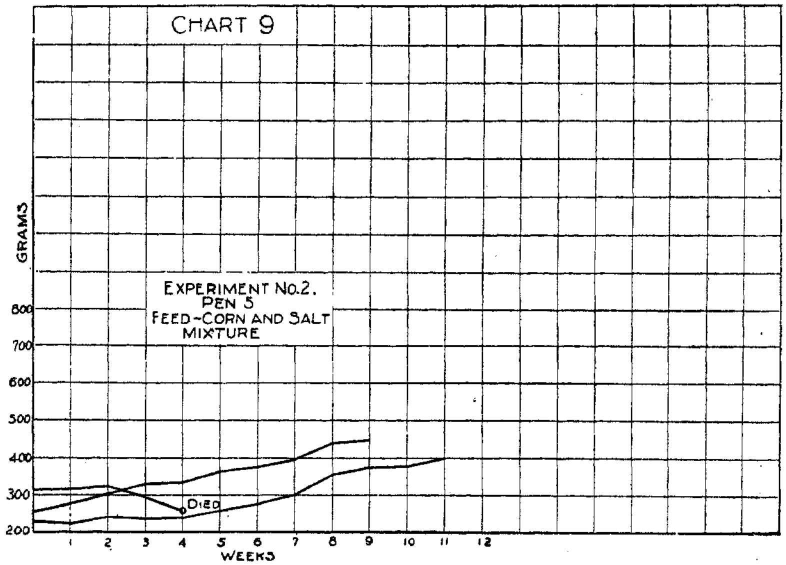


CHART 9
EXPERIMENT NO.2.
PEN 5
FEED-CORN AND SALT
MIXTURE
GRAMS
800
700
600
500
400
300
200
DIED
1 2 3 4 5 6 7 8 9 10 11 12
WEEKS

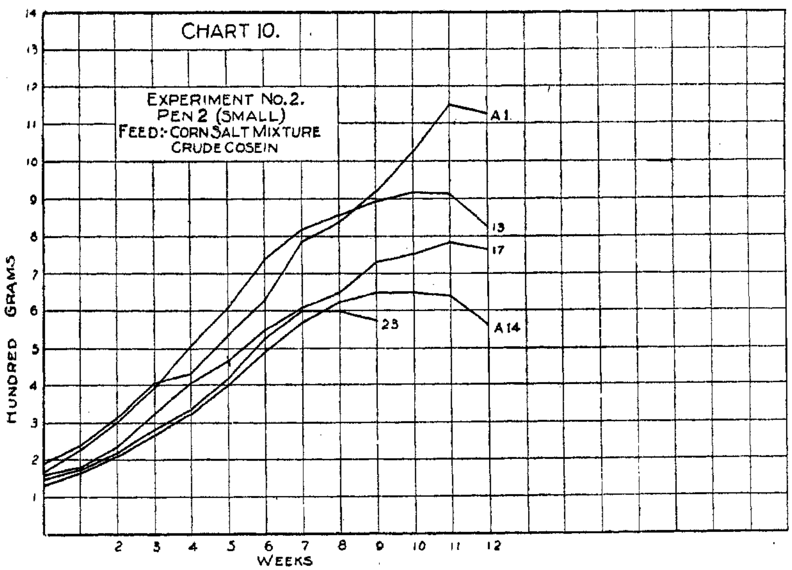


CHART 10.
EXPERIMENT NO.2.
PEN 2 (SMALL)
FEED:-CORN SALT MIXTURE
CRUDE COSEIN
HUNDRED GRAMS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
A 1.
13
17
23
A 14
2 3 4 5 6 7 8 9 10 11 12
WEEKS

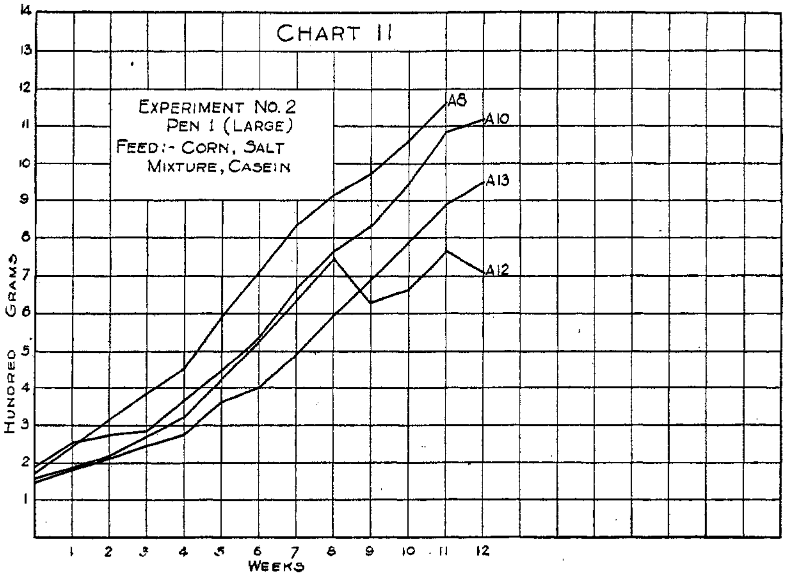


CHART 11
EXPERIMENT No. 2
PEN 1 (LARGE)
FEED:- CORN, SALT MIXTURE, CASEIN
A8
A10
A13
A12
HUNDRED GRAMS
WEEKS

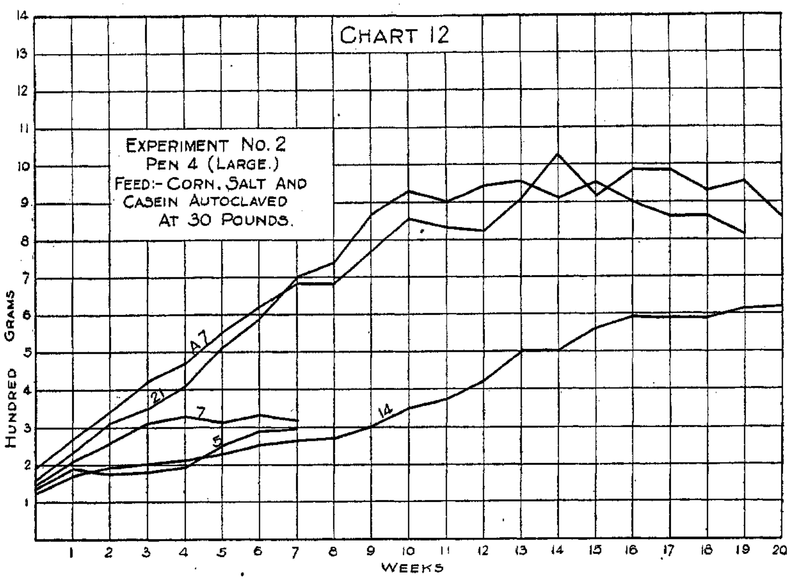


CHART 12
EXPERIMENT No. 2
PEN 4 (LARGE.)
FEED:- CORN, SALT AND CASEIN AUTOCLAVED AT 30 POUNDS.
A7
21
7
5
14
HUNDRED GRAMS
WEEKS

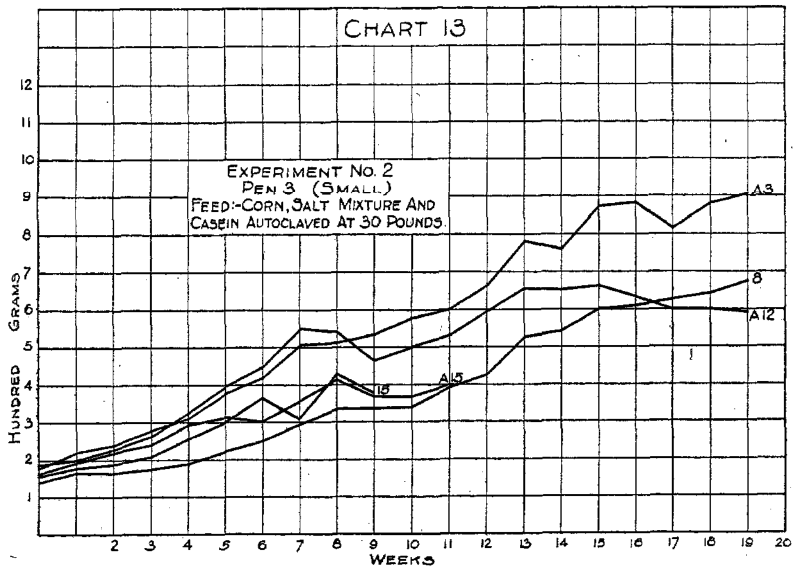


Fig. 4 shows two chickens from the pen receiving corn and salt mixture and casein, and two chickens of the same age receiving casein which has been autoclaved for 2 hours at 30 pounds.

Fig. 5 shows a near view of a chicken receiving the autoclaved casein. This figure shows the eczematous condition of the skin and ruffled feathers common to many of the chickens receiving an inadequate diet. In this case, as in many others, the feather tract on one side of the neck was so drawn that the head was turned around over the back and the chicken was unable to straighten its neck.

The results agree with those of the previous experiment in showing a loss of nutritive efficiency of the casein caused by autoclaving it for 2 hours at 30 pounds pressure. This is true in the pens of both sizes, showing that the lack of exercise was not a determining factor.

EXPERIMENT III.—As pointed out in Experiments 1 and 2, the abnormal growth of young chickens on a diet of corn supplemented with autoclaved casein, may be due to the destruction of some food accessory contained as an impurity in the casein. A third experiment was conducted to determine this

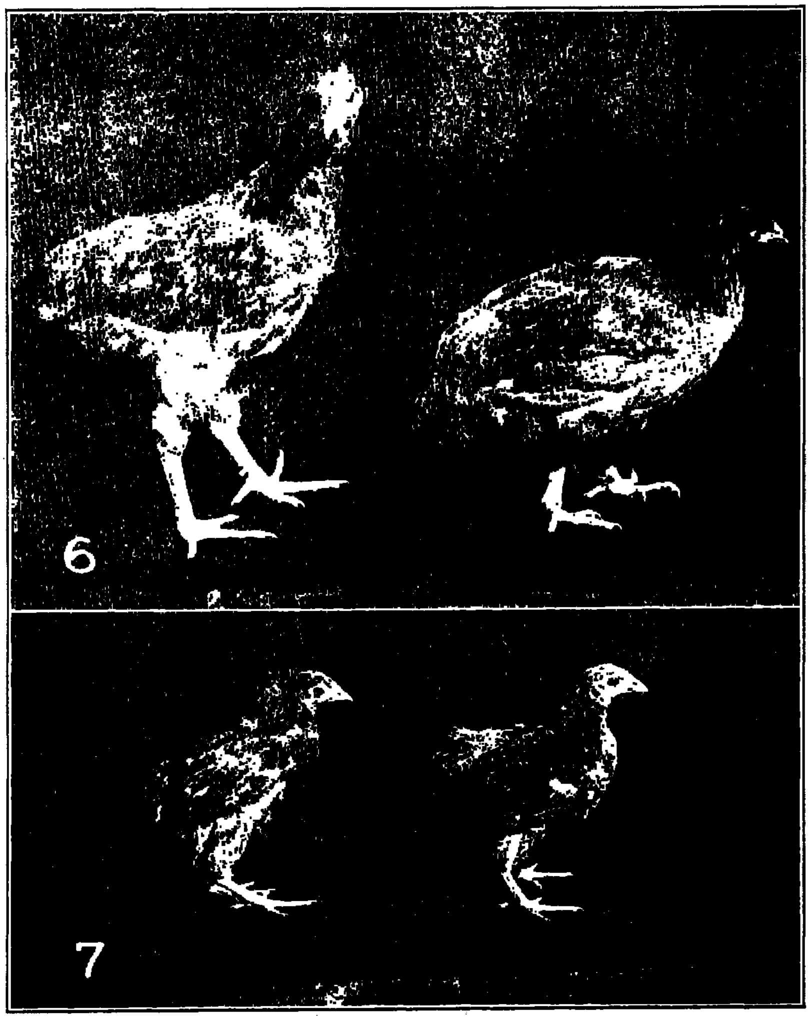
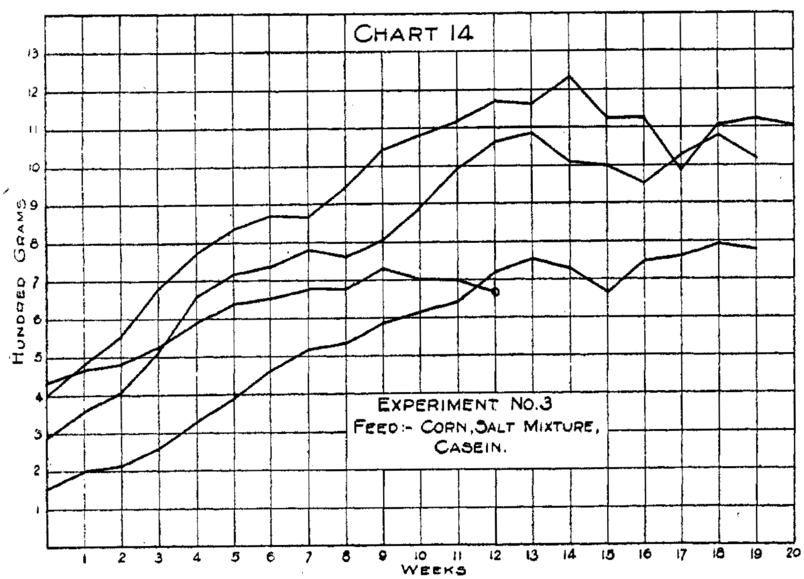


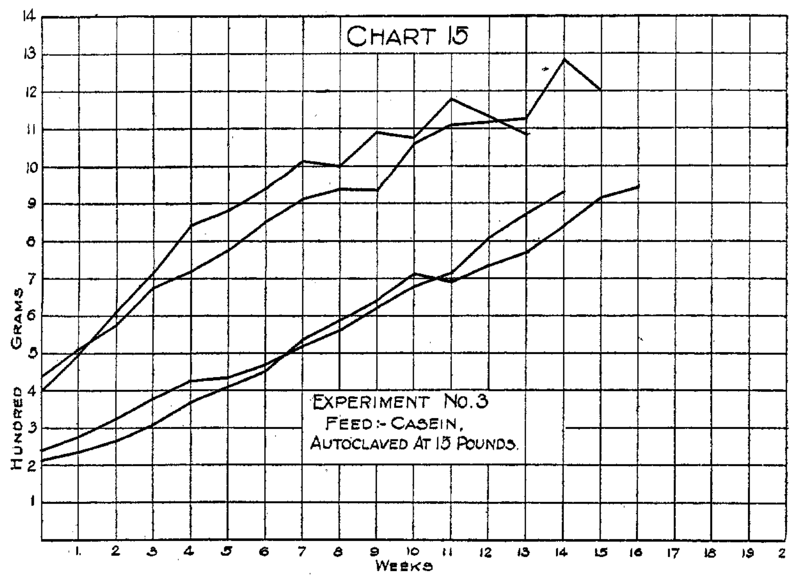
FIG. 4.—No. 6 shows two chickens from Pen 1, Experiment 2, which received a ration of corn, casein, and salt mixture. No. 7 shows two chickens of the same age from Pen 3, which received a ration the same as Pen 1, except that the casein was autoclaved for 2 hours at 30 pounds pressure

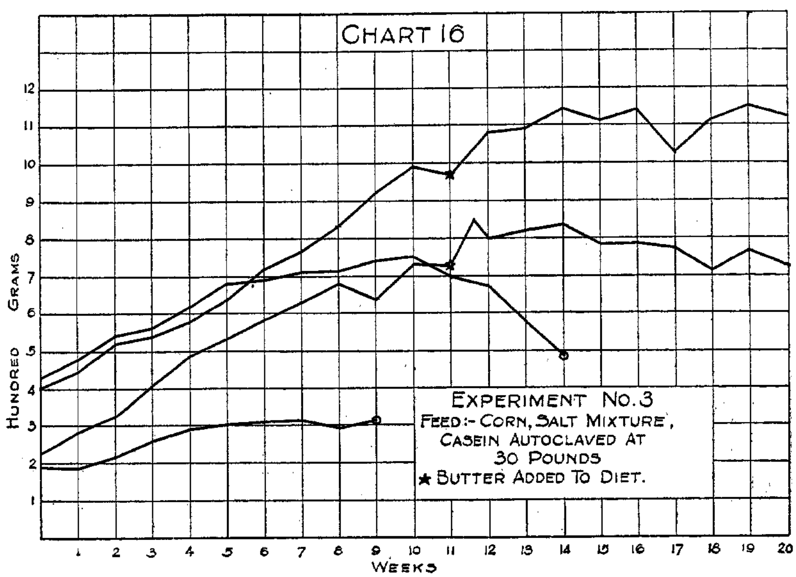
FIG. 5.—A chicken fed corn, salt mixture, and casein autoclaved 2 hours at 30 pounds pressure

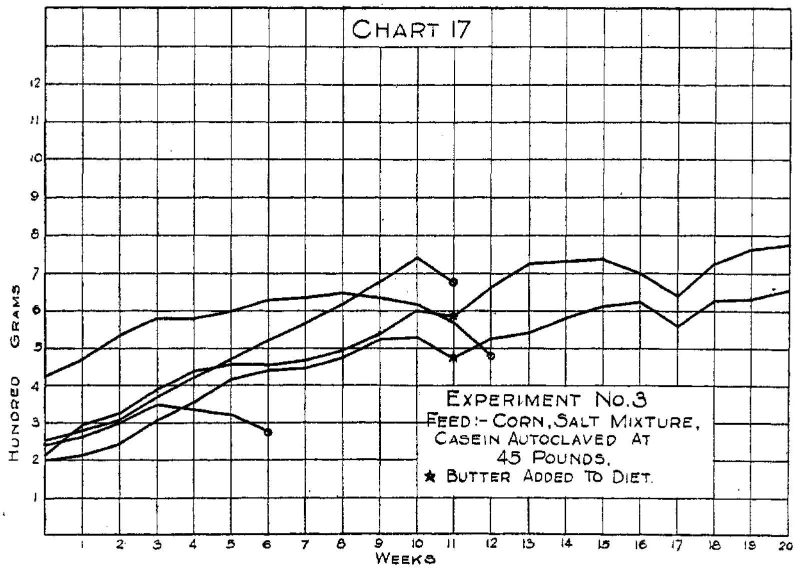
point. There were four pens. The casein in the diet of Pen 1 was not autoclaved. In Pens 2, 3, and 4 the casein was autoclaved at 15, 30 and 45 pounds pressure respectively, the period of treatment being 2 hours in each case. In all the diets, enough casein was added to make 4 percent total nitrogen. Butter fat was added to the diet of two chickens in each of Pens 3 and 4 after the tenth week of the experiment. The results of this experiment are shown in Charts 14 to 17.



As was found in Experiments 1 and 2, casein autoclaved for 2 hours at 15 pounds pressure proved about as efficient as that which had not been autoclaved. That autoclaved at 30 pounds pressure was less efficient and that autoclaved at 45 pounds pressure much less efficient than the crude casein.





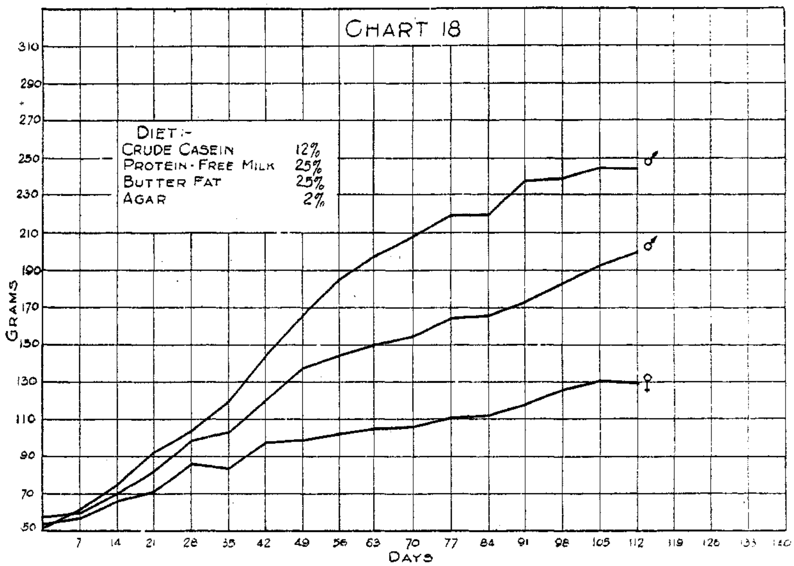


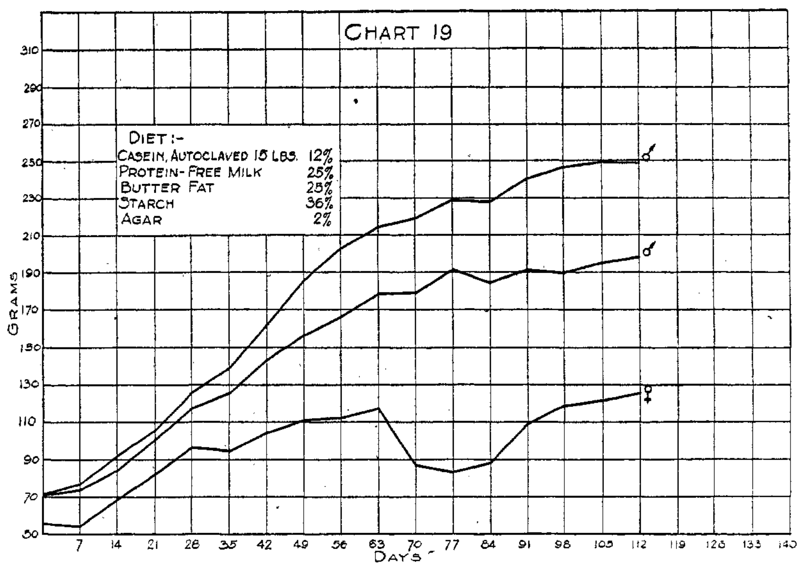
The addition of butter fat to the diet caused only a very slight increase in the rate of the growth. However, a very great improvement in the general appearance of the chickens was noticeable. At the end of 20 weeks the two chickens in Pens 3 and 4 receiving the butter fat were looking just as healthy as those in Pen 1, which received the unautoclaved casein, while the two chickens in these pens which did not receive butter had died.

These results seem to show that the loss of nutritive efficiency of casein as an addendum for corn is due in part to the destruction of some food accessory which is present in butter.

EXPERIMENTS WITH RATS

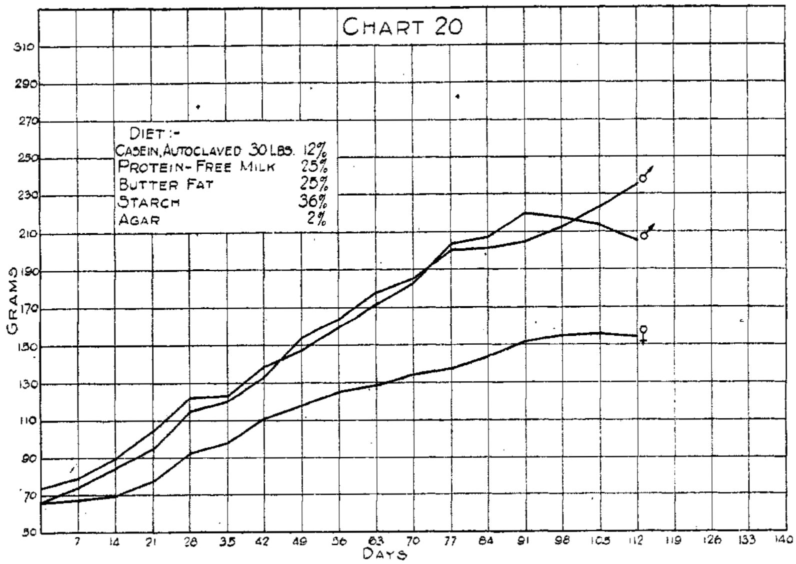
In order to determine whether or not autoclaving casein diminishes its efficiency to promote growth when it is the only protein in the diet, an experiment was conducted with rats, in which the diet was known to be adequate in respect to the food accessories and ash. These substances were supplied by butter fat, "protein free milk" and ash. McCollum (1915) has criticized the use of "protein free milk" in studying the efficiency of protein because it contains a small amount of nitrogenous material. This objection would not appiy in this experiment, as it was not intended to determine the actual efficiency of autoclaved casein, but to determine the relative efficiency of crude and autoclaved casein. The diet in each case contained only 12 percent casein which, as shown by Osborne and Mendel (1915), is below the minimum amount for maximum growth. Hence any benefit derived from the small amount of protein

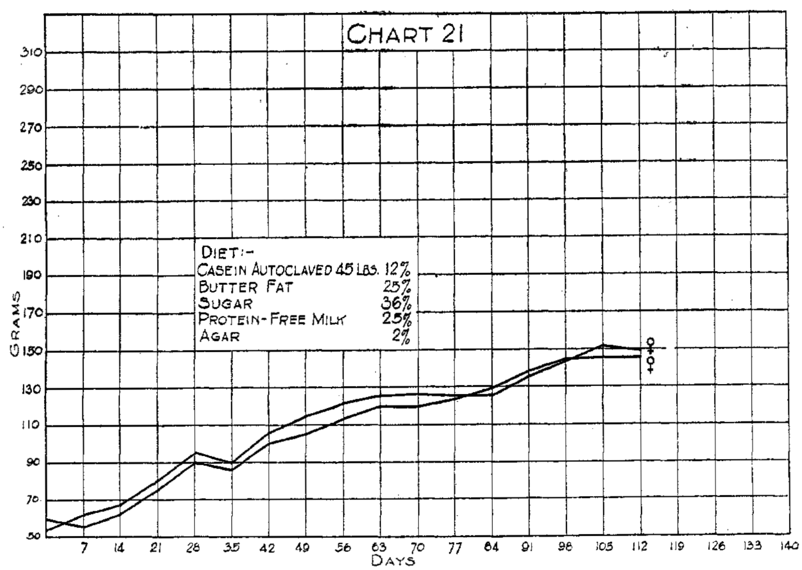




material in the "protein-free milk" would be equal in the diets containing crude and autoclaved casein.

There were four pens. In Pen 1 the casein was not autoclaved; in Pen 2 it was autoclaved at 15 pounds pressure for 2 hours; in Pen 3 at 30 pounds pressure for 2 hours; and Pen 4





at 45 pounds pressure for 2 hours. The results are shown in Charts 18 to 21. No appreciable difference in the rate of growth of the rats in these four pens was observed. As these diets contained only 12 percent of casein, any loss of efficiency would have been manifested by a slower growth.

Since autoclaving does not destroy the efficiency of casein as a protein for growth, its loss of efficiency as an addendum to corn must be due to the destruction of some food accessory which it contains and which is not present in adequate amounts of corn.

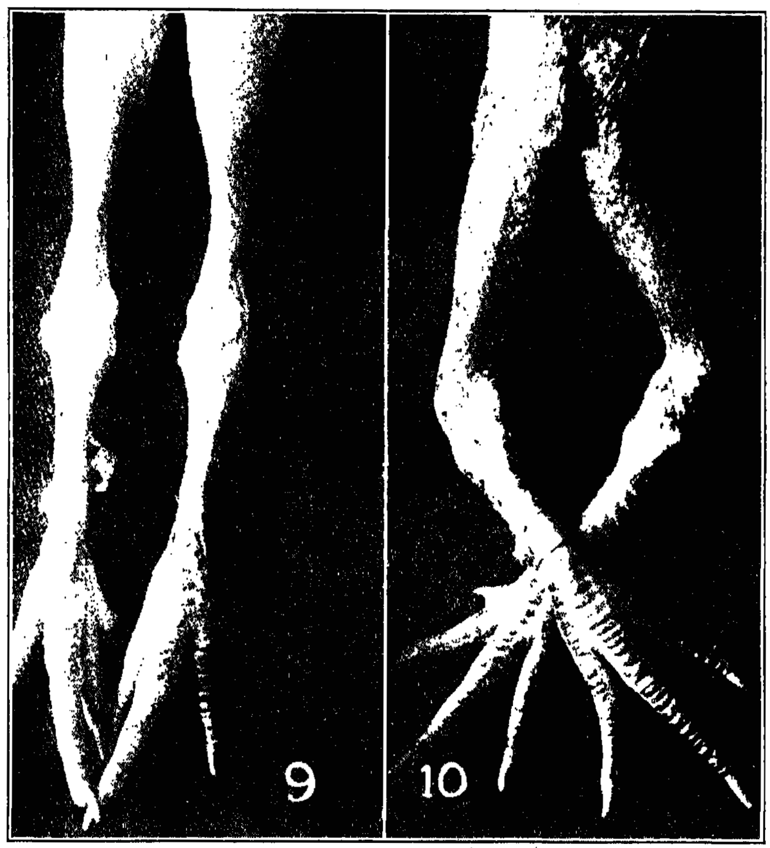


FIG. 6.—No. 9 shows the legs of a chicken which developed normally on a diet of corn, salt mixture and casein. No. 10 shows the legs of a chicken which received the same diet with the exception that the casein was autoclaved for 2 hours at 15 pounds pressure. This autoclaving did not prevent the chicken from growing rapidly, but did cause an abnormal development of the bones

THE EFFECT OF FEED ON THE DEVELOPMENT OF BONES

It is quite common for young chickens to develop weak bones in the wings and legs, especially if they grow very rapidly. This is particularly noticeable in commercial milk feeding where the young chickens grow very rapidly on a diet deficient in ash. The experiments described in this paper were not planned to study the causes of the abnormal development of the bones, but some interesting observations in this connection were made.

In Experiment I, in which chickens were given a diet of corn, casein autoclaved at 15 pounds pressure, and salt mixture, one of them began to show weak legs during the third week. By

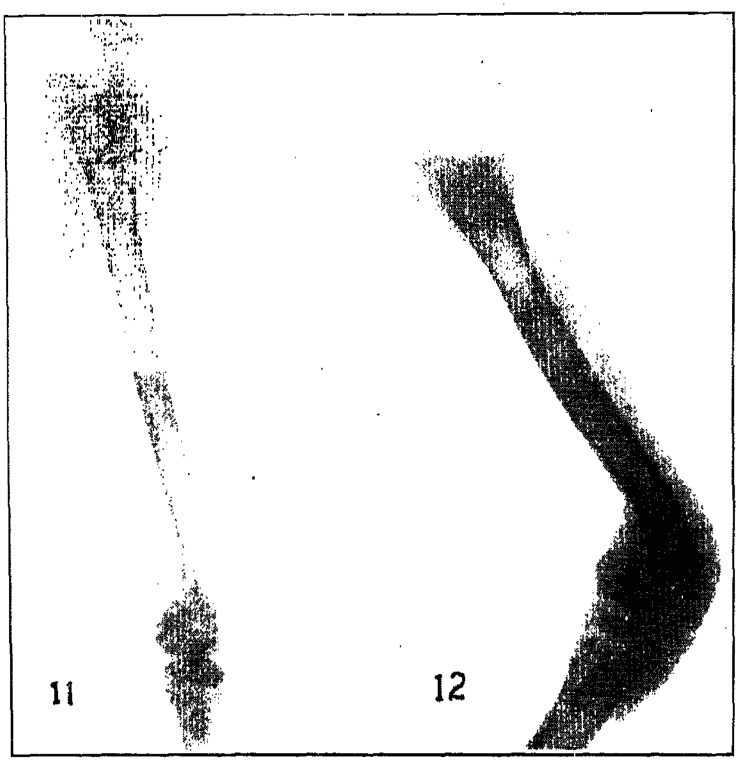


FIG. 7.—No. 11, radiograph of a normal leg of a chicken receiving a ration of corn, casein, and salt mixture. No. 12, radiograph of an abnormal leg of a chicken receiving the same ration as that in No. 11 with the exception that the casein was autoclaved for 2 hours at 15 pounds pressure

the end of the fifth week its legs were very badly bowed, as is shown in Fig. 6.

An X-ray examination was made with the result shown in Fig. 7.

The radiograph (Fig. 7), which compares the leg bone of this chicken with one on a diet in which the casein was not autoclaved, shows that the head of the bone was separated from the shaft. Since all the conditions except the casein were the same, the results seem to show that autoclaving destroyed some substance which was essential to the proper development of the bone. The X-ray shows a very marked separation of the shaft from the head of the bone in the chickens receiving the casein autoclaved at 30 pounds, but as the chickens did not gain weight so rapidly, their legs did not become so crooked.

The breaking strength of these abnormal bones was determined and was found to be much weaker than the normal ones, although larger in diameter. The cross sections of these broken bones are shown in Fig. 8.

In some cases the marrow cavity was filled with a spongy bone material similar to that found in the head of the bone. This shows that absorption had not taken place normally. As stated above, this abnormal condition is perhaps due in some way to the feed, but more experiments must be conducted to determine the factors causing this condition. It will be seen (Fig. 9, No. 19) that the bones of the chicken receiving extracted casein resemble those of the chicken (Fig. 9, No. 17) receiving autoclaved casein.

FIG. 8.—*Cross sections of bones*

No. 13.—Bones from a normal chicken from the Agricultural College poultry farm.

No. 14.—Bones from a chicken confined in a small yard receiving the same feed as the chicken from the poultry farm.

No. 15.—Bones from chicken receiving corn, salt mixture, and casein.

No. 16.—Bones from chicken receiving corn, salt mixture, and casein autoclaved for 2 hours at 15 pounds pressure.

An examination of the bones of growing chickens shows that confinement has but little effect on the development of the bone, for the bones of the chickens running in the poultry yard (Fig. 8, No. 13) are no different from those of the chicken which was confined in the small pen 4 feet square and fed chick feed (Fig. 8, No. 14). The abnormal condition of the other bones must be due to some defect in the feed.

FIG. 9.—*Cross sections of bones*—concluded

No. 17.—Bones from chicken receiving corn, salt mixture and casein autoclaved for 2 hours at 30 pounds pressure.

No. 18.—Bones from chicken receiving corn and salt mixture.

No. 19.—Bones from chicken receiving corn, salt mixture and extracted casein.

No. 20.—Bones from White Leghorn cockerel which had been fed exclusively on corn and salt mixture for 1 year.

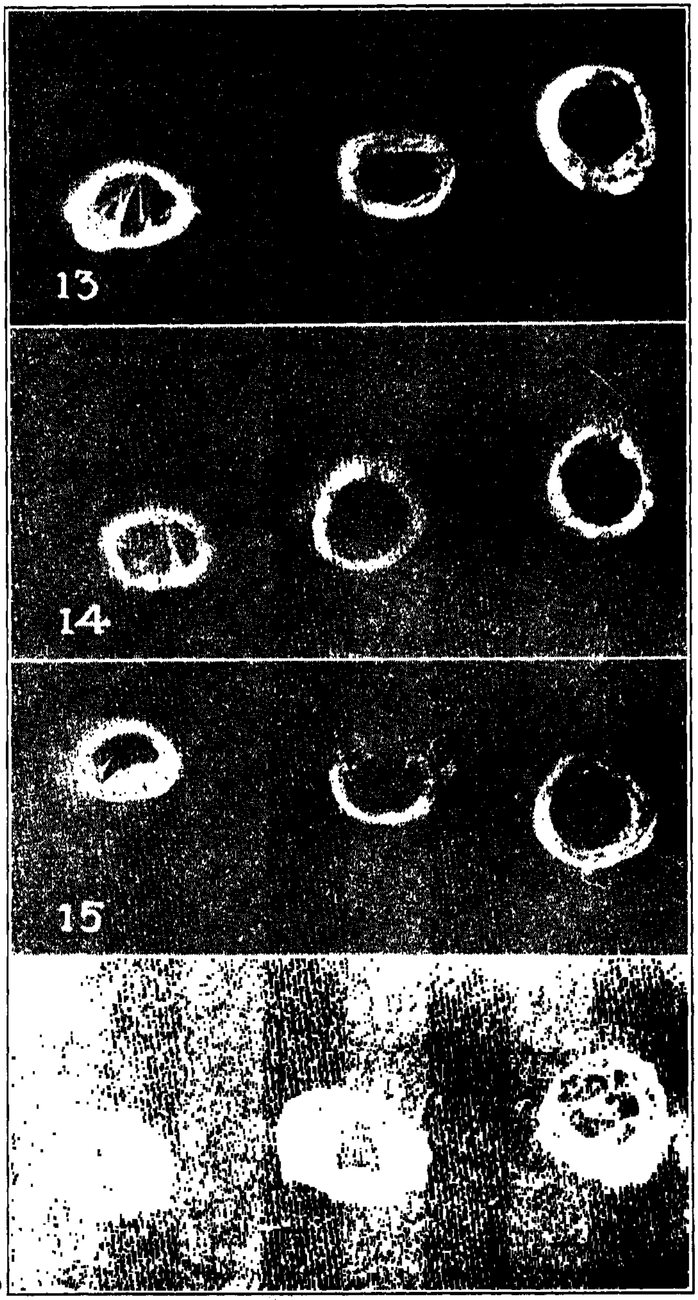


FIG. 8.—Cross sections of bones

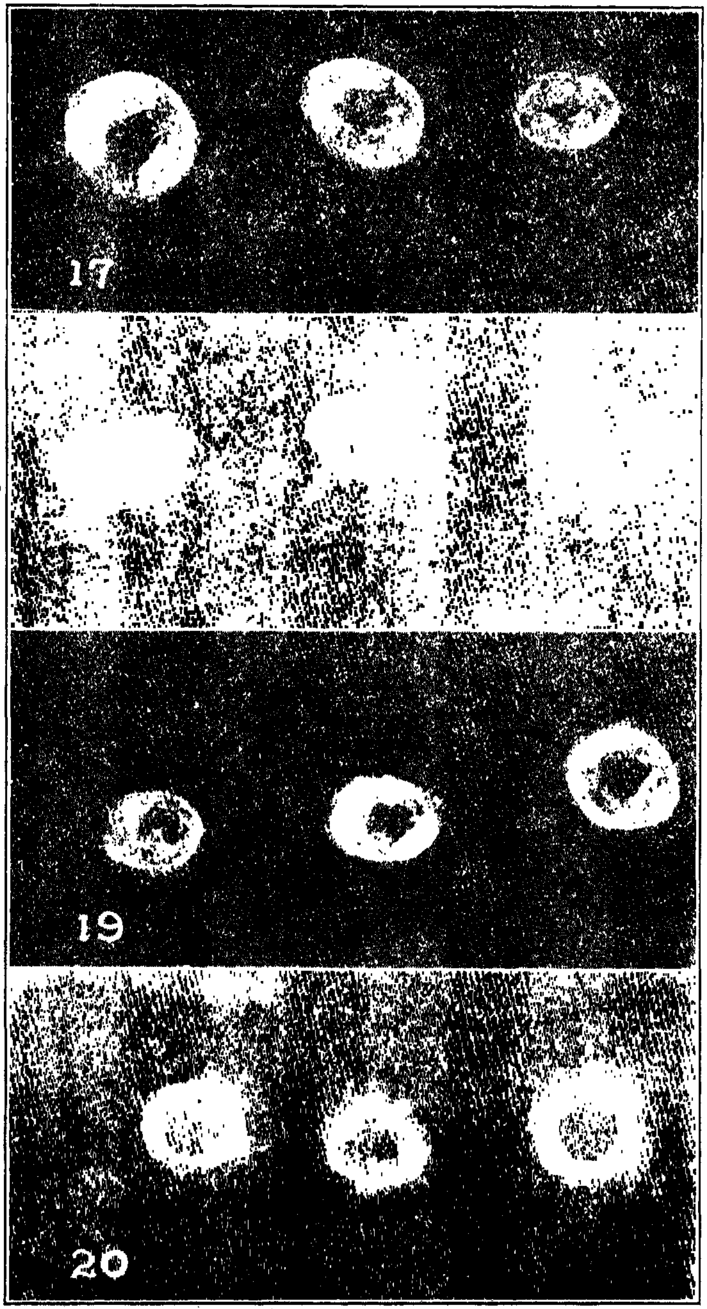


FIG. 9.—Cross sections of bones—*concluded*

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