AN EXPERIMENTAL STUDY OF FAT STARVATION WITH ESPECIAL REFERENCE TO THE PRODUCTION OF SEROUS ATROPHY OF FAT.

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PLATES XXIII AND XXIV.

The experimental study which forms the basis of this paper was originally undertaken to determine whether the lesions of rickets or similar lesions can be produced in growing animals by withholding fats from their food as far as practicable. This inquiry was suggested by the alleged frequently low fat content of the milk of women whose children have grown rachitic on breast-milk, by the occurrence of rickets in children fed largely on condensed milk, which is poor in fat, and by the fact that the clinical indications of rickets are often lessened by a diet rich in fats.

The common pig, Sus scrofa, was the animal chosen for the experiment, partly because of his omnivorous habits and partly because the nutritive vigor of this animal seemed likely to render possible the extension of the necessarily depressing experiment over a long period of time.

CLINICAL SECTION.

Pig I.—The first experiment was begun December 16, 1895, upon a boar about two months old weighing 24 pounds, and which will be referred to here as pig 1. This animal was fed exclusively upon milk furnished by the Walker-Gordon Milk Laboratory, from which the fat had been largely removed by the centrifuge. The composition of this milk as regards fat is remarkably constant, and analysis shows that it contains less than $\frac{1}{4}$ per cent of fat. The milk of the sow normally contains a high percentage of fat, the amount ranging from 8 to 10 per cent. The usual content of cow’s milk skimmed by the ordinary method is about 1 per cent. The milk fed to pig 1, therefore, contained less than $\frac{3}{16}$th part of the fat which is found in sow’s milk, and about $\frac{5}{8}$th part of the fat contained in ordinary skimmed milk. In other respects the milk did not differ greatly in composition from
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that of cow’s milk, the content of milk-sugar being about 6 per cent, that of proteids about 4 per cent.

The duration of the experiment was 51 weeks. The daily quantity of milk allowed first was 1400 cc. This was increased from time to time until at the end of 46 weeks the total daily amount was 4000 cc. The feeding was usually in two portions, towards the end often in one. During the first six weeks the initial weight of the animal was just maintained; then during a period of about six weeks there was a moderate loss, the weight dropping from 24 to 19½ pounds. After this an increase in the food supply was followed by a steady gain in weight, so that 12 weeks after the beginning of the diet the animal weighed 2½ pounds more than at the beginning. 16 weeks from the beginning the weight showed an increase of 5 pounds, in 20 weeks an increase of 7½ pounds, and in 50 weeks an increase of 16 pounds, in all from 24 to 40 pounds. Within a week from the beginning the faeces were observed to be lighter in color and firmer in consistence than when the animal was on a mixed diet containing cereals. Very soon there was distinct constipation and the faeces were passed as small hard spheroidal masses. This constipation continued to the last. The faeces were regularly alkaline in reaction. The quantity of urine passed varied closely with the amount of milk given. The specific gravity varied between 1011 and 1014 in 24-hours’ collections. The reaction was invariably distinctly acid—a notable peculiarity, as the urine of pigs on a general diet is almost always neutral when passed. During the first 8 or 10 weeks the animal seemed to be in every way normal except that he did not gain in weight. After 18 weeks it was observed that the animal’s hair and skin were distinctly drier than is usual in pigs of this age, and that the hair was decidedly more scanty than that of a control pig of the same age. After 30 weeks these conditions were more pronounced, and some weakness of the hind legs was noted when the animal stood. From about this time it was noticeable that the animal lay on the floor of his cage a good deal of the time, and often dozed during the day, though he did not usually sleep. After 50 weeks he was quite unable to stand on his hind legs, which were thrown forward as the animal lay. There was no attempt whatever to use these extremities in walking. Some power remained in the hip muscles. The muscles were flabby but showed no indication of wasting. On the contrary the legs were puffy in appearance, although there was absolutely no pitting on pressure. This condition had doubtless been present some time before it was noted. The knee-jerks were present. The forelegs were weak, but the animal was able to walk by use of them. For a few days before his death the
animal had a rise in temperature, his breathing was rapid and labored and he was cyanosed. When it was evident that death was impending he was killed. An examination of the blood just before death showed the hemoglobin to be reduced to 65 per cent by Fleischl's instrument. The proportion of small red blood cells seemed increased. The proportion of white to red cells was normal. The blood count showed a slight reduction in the total number of red cells to the cubic millimetre.

Pig 8.—A second experiment was conducted with another boar weighing 26 pounds, and referred to here as pig 8. This animal was fed exclusively upon milk of the same composition as that used in the case of pig 1, but was given during the first twelve months of the experiment as much of the food as he was able to take, instead of being limited to a fixed quantity. The quantities consumed by him were considerably in excess of the amounts allowed pig 1—5, 6 or 7 litres per day. Toward the end of the first year the quantity of milk consumed was from 8 to 10 litres daily; from the end of the first year until the termination of the experiment the quantity was reduced to two litres per day. The duration of the experiment was 66 weeks. At the end of 12 weeks the skin was observed to be dry and scaly, and the hairs were scanty. At the end of 20 weeks some weakness of the forelegs was shown in standing, and the animal was inclined to sleep a good deal of the time. After 30 weeks there was marked weakness in all four legs. The appetite remained good. The urine and feces showed the same peculiarities as were noted in the urine and feces of pig 1. From time to time in the course of this experiment bits of skin and subcutaneous fat were removed from the back of the neck in order that their structure might be compared with a piece removed before the animal was put on this diet, and with the skin of pig 1. The weights of the animal were as follows: Nov. 12, 1895, 28 pounds; Jan. 21, 1896, 42 pounds; February 29, 55 pounds; April 21, 62 pounds; Nov. 23, 106 pounds. The gain in weight was thus much greater than in pig 1. On the reduction of the milk to 2 litres daily there ensued considerable loss in weight, and one week before death the animal was quite unable to stand owing to weakness. He was killed by bleeding.

Pig 9.—A third experiment was conducted on a pig weighing 24 pounds, with a view especially to a study of the chemical characters of the urine and feces during the starvation. After 6 weeks the animal was again allowed an abundance of fat, and after two weeks this was again cut off. This animal is referred to as pig 9.

Pig 2.—At the time when pig 1 was put on a fatless diet, another animal, here known as pig 2, of about the same age and weight, was
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placed upon a wholly different diet, in which the quantity of protein and of fat was much reduced, but in which an attempt was made to compensate for their reduction by the use of very large amounts of carbohydrate food, first in the form of Mellin's food, later as unrefined sugar. The results obtained from the study of this animal bear directly on the question of fat starvation, and in some ways the experiment may be regarded as a control to the others. The duration of this experiment was 4 weeks longer than in the case of pig 1, namely, 56 weeks. There are several respects in which a comparison between the two animals is instructive. The urine, instead of being distinctly acid, as was regularly the case with the urine of pig 1, was neutral or alkaline at first, and later slightly acid in reaction. Toward the end of the experiment the urine at times contained sugar in small amounts soon after the administration of the richly saccharine food. The feces, instead of being hard in consistence and light in color, as in pig 1, were usually dark in color and semi-fluid in consistence, and part of the time were diarrheal in character.

More important clinical differences relate to the general condition of the animals. Although there was an even greater retardation of growth in pig 2 than in pig 1, in the former the skin remained soft and apparently well nourished, though a little dry. There was some falling out of the hair, but that which remained was abundant in comparison with the hair of pig 1. The animal appeared fairly well nourished, though small. There were no hemorrhagic spots in the skin. A few days before the animal was killed he seemed rather feeble in all four legs, as shown by difficulty in standing, but he developed no distinct paralysis. There was no swelling of the feet as in the case of pig 1.

To sum up: it was found that a growing pig which lived exclusively on nearly fatless milk for about one year was retarded in growth, suffered markedly in nutrition, and developed a hemorrhagic eruption* and marked weakness of the extremities; that another pig kept on the same diet for 66 weeks developed the same clinical conditions excepting hemorrhagic spots, but in less marked degree; and that a third animal which lived on a diet much restricted in proteids and fats but with a great excess of carbohydrates, though more retarded in growth, suffered distinctly less in general nutrition and apparently in a different manner in several respects.

* It seems not unlikely that certain of the lesions noted in this animal, especially the hemorrhagic ones, were scorbutic in character.
Skin and Adipose Tissue of Pig 1.—The body is poorly nourished; there is a fairly abundant haemorrhagic eruption on the lower part of the abdomen and into the skin of the perineum. Over the upper part of the sternum is an ulcer about 2 cm. square (clearly from pressure). On cutting through the skin it is seen that there is nowhere any subcutaneous fat; in place of this is a homogeneous, translucent layer of gelatinous-looking material, somewhat less thick than the layer of subcutaneous fat in a normal pig of the same size. On the back of the neck, near the median line, where the fat is normally very thick, this material forms a layer about 22 mm. in thickness. It may be stated here that in all parts of the body where fat is normally present a similar replacement of it by this material is evident, as about the heart, about the kidneys, between the muscles, in the omentum and elsewhere. At first sight the appearance of the material suggests that of oedematous connective tissue, but the substance appears quite homogeneous and yields no fluid on being cut into small pieces and compressed. This replacement of fat by the material described is the most striking and important pathological change noted in this animal. The microscopic characters of the skin are normal. The subcutaneous fat-cells are found to be so transformed as to be almost unrecognizable. The gelatinous layer consists chiefly of spheroidal and ovoidal cells (Plate XXIV), the average diameter of which is from one-third to one-sixth that of normal fat cells (Plate XXIII). The cells consist of a cell membrane, frequently irregular in outline, containing a homogeneous substance which stains rather faintly with haematoxylin and most basic aniline dyes. Each cell contains a nucleus which stains deeply with haematoxylin and resembles that of a young fat cell. These nuclei are not applied closely to the cell membrane, but may occupy any portion of the cell body. The intranuclear structure was not studied. Often the nucleus is surrounded by a small or considerable amount of finely granular material. Not infrequently what appears to be a vacuole of considerable size is found in the cell body. Sections from material subjected to the action of osmic acid, before further hardening and embedding, show no evidence of the presence of fat—they are wholly unaffected by this reagent. Running in from and continuous with the connective tissue of the skin are bundles of various sizes formed by connective tissue fibres. Frequently the smaller bundles separate into the individual wavy elastic fibres of which they consist, and these pass in all directions between the cells.* The

*The elastic fibres are very abundant, but this is normally the case in the skin from the region of the ligamentum nuchae.
cells do not lie close together as in normal fat tissue, but are separated from one another in places. Their appearance suggests that the intercellular spaces may have been occupied by some material which has been lost during the process of hardening or embedding. Between the cells with unbroken cell membranes sometimes lie processes which may consist of broken cell membrane. The capillaries are fairly numerous. Some of them have blind ends. Unfortunately the material from pig 1 was not examined fresh.

Before describing other changes noted in pig 1 it will be convenient to refer to the changes found in the skin in pig 8 and in pig 2.

Subcutaneous Fat in Pig 8.—In this pig, skin from the back of the neck was removed from time to time. After nine weeks it is noticeable that there is some shrinkage of the fat cells, and that the cells in places are irregular in form. After fourteen weeks the fat cells are shrunken to two-thirds or one-half of their normal size; the outline of the cell membrane is often exceedingly irregular; the cell contents consist of larger and smaller droplets of fat which stain well with osmic acid, and it is noticeable that some of the nuclei are no longer in apposition with their cell walls. Twenty weeks after the beginning of the diet the fat layer appears pale. Although it does not look quite like normal fat, it does not appear gelatinous. The fat cells show more reduction in size than in the preparations referred to above. This is more pronounced in some lobules than in others. A few cells are seen which are not more than one-quarter or one-fifth the average diameter of normal cells. In preparations cut from pieces immersed in a 1 per cent solution of osmic acid the fat cells nearest the edge of the section have stained like normal fat cells, but there is a line of cells a little farther from the edge to which the osmic acid has penetrated less freely. Here the contents of the cells have stained only in spots, corresponding to the fat droplets already referred to. Between the droplets is seen in places a very finely granular or homogeneous material which does not stain with osmic acid, but tinges faintly with hematoxylin. This certainly is not fat, and it is possible that it is the accumulation of such material in larger amounts both in and between the cells which gives rise to the gelatinous appearance noted in pig 1. The nucleus in many of the cells lies well within the cell body. The connective tissue which lies between the groups of cells shows a distinct inclination to the separation of the individual fibres of which it consists—an unraveling, as it were, similar to but much less pronounced than that observed in pig 1.

The only additional change observed in the subcutaneous fat of this animal at the end of 60 weeks is a further considerable reduction in the
size of the fat cells. In some places the subcutaneous fat has almost wholly disappeared; in most places it is from \( \frac{1}{3} \) to \( \frac{3}{4} \) in. in thickness. Nowhere has there been any replacement of fat by distinctly serous material. A majority of the fat cells are simply much atrophied, but a few show the alterations described as present after the lapse of 20 weeks.

Subcutaneous Fat in Pig 2.—An examination in this case shows that the skin itself is normal in structure. The subcutaneous layer of fat is much reduced in thickness, being only about one-quarter the thickness of the fat layer from the corresponding portion of the back of the neck in a normal pig of the same age. The gelatinous appearance which was noted in the skin of pig 1 is wholly wanting, but the fat is perhaps a little paler in tint than that of normal animals. Minute examination shows that the fat cells are somewhat reduced in size, perhaps to two-thirds or three-quarters of their normal diameters. The contents of the cells are everywhere homogeneous; nowhere is there to be seen the breaking into fat globules noted in the sections from pig 8. The cell contents stain faintly with haematoxylin. With osmic acid they stain exactly like normal fat cells. The connective tissue separating groups of fat cells is quite normal in appearance. Between the individual fat cells are more nuclei than are normally present in an animal 15 months of age, but not more than are seen in the subcutaneous fat of pigs a few weeks old.

The appearances, then, are chiefly those which we might expect to find as the consequence of the arrest of fat formation in a young animal. The reduction in the size of the cells is doubtless to be regarded as the result of a process of simple atrophy. Notwithstanding the long period over which the experiment extended, there is nothing to suggest even the beginning of the transformation which was noted in pigs 1 and 8.

Examination of Pig 1 continued.—On opening pig 1 much straw-colored fluid is found in both pleural cavities, in the pericardium, and in the peritoneal cavity. The parietal pleura on both sides is the seat of a moderate number of small superficial hæmorrhages; the lower lobe of the right lung is partially consolidated, and on section presents numerous larger and smaller infarctions. On the pleural surface over the consolidated area is a thin layer of fibrin. The base of the left lower lobe is congested, but there is no consolidation. Microscopically the consolidated lung presents numerous infarctions with surrounding pneumonic consolidation. The heart is large, pale in color, and flabby in consistence. The weight of the heart is 232 grammes. That of a normal animal of the same age, and weighing
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five and one-half times as much, is only 358 grammes, or about one-half as much more. On the anterior surface on either side of the coronary artery are several hemorrhages of moderate size, extending about one-quarter of an inch into the myocardium. In place of the subpericardial fat usually found is a layer of the gelatinous tissue above described. On section the myocardium is pale and the wall of the left ventricle is notably thickened. The valves are normal. There are several small endocardial hemorrhages in the left ventricle. The coronary arteries were not examined. Microscopic examination shows that many of the muscle fibres of the left ventricle are the seat of slight granular degeneration. In places there are small areas of richly cellular connective tissue. The liver is normal in appearance and consistence. The gall-bladder is distended with light-colored bile. The bile duct is pervious. The histological appearances of the liver are normal. The mucous membrane of the stomach is normal in appearance. In the cardiac portion there are a few hemorrhagic spots in the mucosa, and one small superficial ulcer. The mucous membrane of the large and small intestine is normal in appearance. The pancreas is normal in consistence, yellowish in color and disproportionately large in comparison with the body weight. Histologically it appears to be normal. The spleen is small, rather pale, and normal in structure. The kidneys are surrounded by a considerable amount of hyaline gelatinous material of the same character as that beneath the skin and representing altered adipose tissue. The kidneys are disproportionately large in comparison with the body weight. The capsules strip off readily. The surfaces are smooth, slightly paler than normal; the striations are almost obliterated and the cut surface is studded with numerous minute hemorrhages. The chief histological changes observed in the kidney are as follows: The epithelium of the tubules is everywhere the seat of granular degeneration, the changes being slight but distinct in the collecting tubes and in Henle's loops, and considerable in degree in the secreting tubes. The cells of the secreting tubes are somewhat swollen and in some cases desquamated. Many degenerated cells fill the tubes. In other cases the contents of the tubes consist of the nuclei of degenerated cells and a granular detritus. Frequently the secreting tubes contain hyaline material which stains with eosin. Nowhere do the tubules seem much dilated. The tufts are somewhat swollen and usually occupy the entire capsular space. They appear more richly cellular than normal. The epithelium covering the tufts is in places swollen and granular, that lining the capsule appears normal. The capillaries of the tuft generally appear compressed. The vessels everywhere seem
normal. No alterations can be detected in the connective tissue. In short, the appearances are those of a chronic parenchymatous degeneration. The adrenals are normal in consistence but are distinctly increased in size, their weight being as great as in a normal pig whose body weighs more than five times as much as that of pig 1. On section the cortex appears thickened and the medulla is abnormally dark, suggesting the presence of hemorrhage, but this view is not confirmed by microscopic examination. The radially disposed columns of cells in the cortex appear swollen, and in places the cell bodies are less granular than normal. The nuclei seem unchanged. In the medullary portion the ganglion cells have become greatly swollen and in some cases are almost wholly replaced by large vacuoles. Through an oversight the thyroid was not removed.

The knee-joints are found to be filled with bloody and viscid synovial fluid. The bones of the skull are porous, very brittle and distinctly thinner than those of a normal animal of the same size. The femurs are about normal in weight. The bone marrow in each femur is replaced wholly by material which has the appearance and consistence of a blood clot. Unfortunately this material was not successfully hardened, and no satisfactory examination was made of it. The remaining long bones were not examined.

The central nervous system was not examined histologically. Of the skeletal muscles pieces only of the quadriceps femoris were examined histologically. Sections of the muscle show that a majority of the muscle fibres have undergone a marked reduction in size. While there is an apparent increase in the sarcolemma nuclei, there is probably no actual increase in their number. A few fibres are reduced to a width not greater than the diameter of their nuclei. The striations of the fibres are as a rule well preserved. The fibres stain well with eosin, and on transverse section some of them have a vitreous appearance and look as though broken into large fragments. There is no increase in connective tissue. The intermuscular fat shows the same peculiarities that have been noted in the case of the subcutaneous fat. All the visible vessels show an increase in the thickness of their walls, which is in some cases very marked and implicates chiefly the muscular coat.

The post-mortem notes on pig 2 are as follows: There is little or no subcutaneous fat; the lungs are normal; the heart is normal in size, color and consistence; the muscles are a little soft; the liver is normal in appearance and consistence; the pancreas is rather hard; the kidneys are normal; the striations of the cortex are very plain. The femur, cut longitudinally, shows the development of bone to be almost exactly the
same in degree as in the case of pig 1. In thickness and consistence
the bone is not perceptibly different from that of pig 1. The marrow
adjacent to the compact bone is red in color; it has the appearance pre-
presented by all the marrow of pig 1, whilst the remaining and greater
part of the marrow, that occupying the central portion, is of the normal
light pink color. The hæmoglobin was estimated immediately after
death. The reading with Fleischl's instrument was from 100-105 per
cent.

Examination of pig 8.—The animal is considerably emaciated. The
subcutaneous fat is everywhere either entirely absent or greatly reduced
in amount. The muscles are normal in color and consistence. The
pericardium contains almost two ounces of clear straw-colored fluid.
The heart is flabby in consistence and pale in color. Hæmorrhages
into the heart muscle are not noted. The liver, spleen, pancreas and
kidneys appear normal. The epi- and pericardial fat presents the same
gelatinous appearance observed in pig 1. The same change is very
pronounced in the adipose tissue about the kidneys and the ureters and
in the omentum. Microscopic examination shows that the atrophic
adipose tissue about the heart, kidneys and elsewhere presents essen-
tially the same appearances as those already described as occasionally
present in the adipose structures of pig 1. The only difference worthy
of mention is that there is visible an abundant homogeneous intercellular
matrix, which probably consists of an albuminous serous material.
Aside from the alterations in the adipose tissue the following changes
are noticeable. The skeletal muscles show granular degeneration of the
muscle fibres in places; other fibres exhibit different grades of simple
atrophy. In the cortex of the pancreas are numerous hæmorrhages of
moderate size, and in places free brown granular pigment. In some
areas the epithelium is the seat of granular degeneration. The secret-
ing tubes of the kidney show marked granular degeneration of the
epithelium; they contain some granular debris and fibrin, and some
hyaline and granular casts. The epithelium of the loops and collecting
tubes shows granular degeneration; the tubes contain much hyaline
material and some granular casts. The capsular epithelium is swollen
and slightly degenerated. Sections from the motor cortex stained with
eosin and hæmatoxylin and by Nissl's method show degenerative changes
in a considerable proportion of the nerve cells. The outer layers of the
cortex are oedematous. The cortex of the frontal region shows similar
changes. In several places there are small hæmorrhages into and be-
neath the pia of the central and frontal convolutions.
PATHOLOGICAL CHEMISTRY.

In the case of pig 1 an endeavor was made to determine the nature of the subcutaneous gelatinous material which has been described. With a view to determining the presence of mucin the tissue was extracted with lime water, mucin being soluble in dilute alkalies. It was found that on the addition of dilute acetic acid to this extract there occurred a considerable flocculent precipitate. Up to this point the behavior of the material is that of a mucin-containing substance. When, however, a 2 per cent solution of acetic acid was added to the precipitate the latter was again dissolved. In this respect the extracted material no longer corresponds to the reactions of mucin. Its solubility in a 2 per cent solution of acetic acid is a characteristic which removes it from the group of mucins, which are not soluble in an excess of acetic acid. Moreover, on decomposition with dilute sulphuric acid it failed to yield a reducing substance such as is obtained in the case of mucin. The mucins are glucosides, that is, compounds of a true proteid (generally a globulin) with animal gum, which by treatment with dilute sulphuric acid are converted by hydration into a reducing substance. On the other hand the lime water extract gave the characteristic xanthoproteic reaction and the reaction with Millon's reagent. This is conclusive evidence that the body under examination is not, strictly speaking, a mucin, but is a true proteid, and probably places it in the group of phosphorus-containing proteids known as nucleo-albumins. Unfortunately the phosphorus content of the material was not determined. Positive proof that it is a nucleo-alumin is therefore wanting.

Before discussing the subject of fat atrophy we may pass to the consideration of some nutritive disturbances which have been noted in the animals that were subjected to fat starvation. As already stated, there was some reason to think that fat starvation might lead to the production of rickets or a condition resembling rickets. For this reason attention was paid to the study of the salts of the urine and faeces, and the results noted were of sufficient interest to induce a continuation of this class of observations even after it became clear that the lesions produced were not those of rickets.

Looking first at the urine, we find that in the case of pig 1 on Dec. 24, 1893, one week after the beginning of the fatless diet, the urea excreted during the 24 hours was 8.047 gm., whilst the $P_2O_5$ excreted in the same period was 1.175 gm., the ratio of $P_2O_5$ to urea being 1 to
6.8. The ratio in health, on a milk diet, varies from about 1 to 6 to 1 to 14. On Jan. 4, 1894, the urea was 18.142 gm., the P₂O₅ 1.190 gm., with a ratio of 1 to 15.2. On January 30 the urea was 30.895 gm., the P₂O₅ 1.023 gm., ratio 1 to 32.4. On April 20 the urea was 25.039 gm., the P₂O₅ 1.065 gm., ratio 1 to 23.5. Finally on Nov. 24, 1894, a few days before the completion of the experiment, the urea was 38.626 gm., the P₂O₅ 0.759, ratio 1 to 50.9. Looking these figures over it will be seen that there was a very considerable increase in the amount of urea excreted between the beginning and end of the experiment, an even greater increase than was to be expected in a young and growing animal. But while this occurred in the urea excreted there was not a corresponding increase in the excretion of P₂O₅. Far from there being any increase in the absolute amount of P₂O₅ excreted, the figures indicate an inclination to an absolute decrease. Under ordinary conditions both in pigs and in man the excretion of P₂O₅ shows a rough correspondence with the urea excretion in any fluctuations that may occur. In the diet of pig 1 the P₂O₅ of the milk was increased proportionately with the proteids of the milk. Hence we should expect to find a more than ordinarily close correspondence between the excreted urea and the P₂O₅. On the contrary we have ratios fluctuating from 1 to 6.8 to 1 to 50.9.

The observations on pig 8 were unfortunately not begun until the fatless diet had been continued for nearly 8 weeks. There is lacking, therefore, a study of the early period in which it is likely that the most pronounced deviation from the normal ratio of P₂O₅ to urea might be expected to occur. The results are, however, not without interest. Between January 9 and April 8, the period of observation, the urea fluctuated irregularly between 90.38 gm. and 42.64 gm. daily, whilst the P₂O₅ varied from .71 gm. to 1.93 gm. The ratios fluctuated irregularly between 1 to 16.70 and 1 to 48.77, with no material difference between the ratios at the beginning and at the end of the period, but with perhaps an inclination to lower ratios at the end than at the beginning. The striking feature of the observations is the high ratios throughout, or in other words the small amount of P₂O₅ in proportion to the urea.

An examination of the ratios in the case of pig 9 leads one to suspect that in pig 8 there may have been a larger excretion of P₂O₅ at the beginning of the fatless period than later. In this case the first observation on the urine was made on February 26, 1896, when the animal had been for four days on centrifugalized milk. The urea increased irregularly, but on the whole gradually, from 4.86 gm. on Feb. 26 to 29.47 gm. on April 11 and 30.32 gm. on April 13. During this time there was an irregular but relatively slight increase in the P₂O₅, so that
the ratios increased from 1 to 10.94 at the beginning to 1 to 32.40 at the end of the period.

We thus have records of three animals fed on nearly fatless milk, in all of which the excretion of $P_2O_5$ is very distinctly lower than in animals fed on milk containing a natural proportion of fat; in two of these cases the study of the urine was begun sufficiently early in the experiment to permit of the detection of a marked fall in the excretion of $P_2O_5$ as a result of the continuation of the fatless diet.*

In the milk on which these animals were fed, which has been shown to be remarkably constant in composition, it is presumable that the $P_2O_5$, present chiefly in salts of Ca and Mg, bore a fairly constant relation to the N present in the proteids of the milk. The N of the urine and the $P_2O_5$ of the urine are admitted to be ultimately derived from the food ingested. How then are we to explain the fact here noted that the $P_2O_5$ excretion did not keep pace with the N excretion? The $P_2O_5$ unaccounted for must be assumed either to have been retained in the body, probably as the Ca and Mg salts of growing bone, or to have been lost through the feces owing to defective absorption. As the latter explanation was thought to be more probable it was determined to study the effect of adding a considerable amount of fat in the form of suet to the usual quantity of fatless milk consumed by pig 9. One hundred grammes of suet, therefore, were given daily with the milk. The feeding remained the same in all other respects. The effect on the urine was immediate and striking. The absolute amount of the $P_2O_5$ increased rapidly to a somewhat higher average than any previously observed in the course of this experiment, in spite of the fact that the absolute urea excretion fell distinctly. The result was an immediate return to the ratios normally observed in pigs on a mixed diet. This may be looked upon as strong evidence in favor of the view that the low $P_2O_5$ excre-

* The fall in the absolute amount of $P_2O_5$ in pig 1 may perhaps be explained by the fact that the quantity of milk received by him was relatively small. Pigs 8 and 9 show no absolute fall in $P_2O_5$. Pig 8 was given an unlimited quantity of milk, pig 9 was on a fixed amount, but this was considerably larger than in the case of pig 1.
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tion observed in these experiments is the result of defective absorption of phosphates from the intestine. This assumption is further reinforced by a study of the excretion of the salts through the feces. In pig 1 we find that the ash of the feces took on an increase from 16.96% of the solid matter of the feces on Dec. 24, 1893, to 47.48% on Jan. 13, 1894, to 49.92% on April 10 and 41.33% on Oct. 5. The first figure is within the normal limits on a mixed diet; the remaining percentages are all distinctly high. In pig 8 the figures representing the percentages of the salts are all very high, ranging as they do from 42.53% to 70.89%. In this case the percentages are only a little higher at the end of the period of observation than at the beginning, but it should be remembered that the analyses were not begun until the animal had been nearly 8 weeks on a fatless diet. In pig 9 again it is found that the percentages of the salts run high throughout (from 36.16% to 54.95%), but in this case the observations were begun two weeks after the beginning of the experiment, and there is a very evident gradual increase in the percentages of the salts lost by way of the bowel. It is interesting to note in this connection that on the addition of fat to the diet of pig 9 there was a prompt fall in the percentage of salts passed with the feces. Of course the mere fluctuations in the fecal fat would account for a considerable fluctuation in the percentage of ash, but not for the marked differences here noted. It is further noticeable that the percentage of $P_2O_5$ and of Ca of the total salts shows a slight progressive increase in both pig 8 and pig 9, which of course corresponds to a considerably greater increase in the absolute $P_2O_5$ and CaO which is lost through the feces. The results obtained make it clear that the presence of fat in the diet exerted an important influence on the absorption of salts, especially those of $P_2O_5$ from the intestine, in the animals experimented upon. They show, at least in the case of pigs, that fat starvation entails a marked diminution in the absorption and in the renal excretion of $P_2O_5$. Whether the same relationship holds good generally in the higher mammals cannot be stated, but it seems likely that it is very generally operative as a physiological principle. I have several times noted the presence of an unusually low $P_2O_5$. 
content of the human urine when the quantity of fat ingested was much restricted. This suggests that the same relationship applies in the human subject. At present I know of no satisfactory explanation of this interdependence.

Whether the deficient supply of salts to the organism is a factor in producing the fat atrophy here described must be regarded as exceedingly doubtful. It is much more likely that its chief effect is to produce the striking retardation in the development of the skeleton which was noted in pigs 1 and 8. Of interest in this connection is the fact that the analysis of the solids of the femur gave essentially the same percentages of $P_2O_5$, CaO, and of ash as the femur of a normal pig of the same age but of much greater skeletal development.

There are other features of interest in the comparison of the analyses made of the various organs of pig 1 with those made upon the organs of a normal animal, but I shall here mention only a few of the more striking results. Thus it is notable that the percentage of fat in the femur of pig 1 was only .48% of the solids, whilst that in the femur of a normal pig was 2.85% or almost six times as much. The percentage of fat in the femur of pig 2, the control animal, on a preponderating carbohydrate diet, was 8% of the solids or sixteen times as much as in pig 1. These figures well illustrate the general impoverishment of the body in fat. In the case of the liver the reduction is much less pronounced. The ether extract from the liver of pig 1 was 8.9% of the solids, from pig 2 it was 12.2%, and from a normal animal 12%. The preservation of so considerable an amount of fat in the liver is in accord with the experiments of Noël Paton, which showed that in pigeons subjected to a fast of four days there was no reduction in the normal amount of fatty acids in the liver, although any excess of fat stored in the liver was rapidly reduced to normal.*

*Probably the object of this storing of fatty acids (as suggested by Paton) is to provide the necessary material for the synthesis of lecithin by the combination of phosphorus with fatty acids. The nucleins of the cells are constantly breaking down, and the store of fatty acids seems necessary to hold the $P_2O_5$ which results from this katabolism. See D. Noël Paton, On the Relationship of the Liver to Fats, *The Journal of Physiology*, xix (1896), 167.
It is also noteworthy that notwithstanding the very great reduction of fat in the body, the lecithins of the brain, which are phosphorized fats, were preserved in their normal proportions. This is shown by the following figures:

<table>
<thead>
<tr>
<th></th>
<th>Pig 1</th>
<th>Pig 3 (normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithins of grey matter,</td>
<td>2.6%</td>
<td>2.9%</td>
</tr>
<tr>
<td>Lecithins of white matter,</td>
<td>5.2%</td>
<td>4.3%</td>
</tr>
</tbody>
</table>

The chief features of interest in the chemical study of the blood were the fact that in pig 1 the serum globulin was markedly reduced in proportion to the serum albumin, and that the urea was considerably below the normal percentage.

The most obvious, and perhaps the most interesting, consequence of the foregoing experiments in fat starvation is the universal serous or gelatinous change in the subcutaneous and other fat depositories. From what has already been said of the microscopical and chemical characters of this altered adipose tissue in pigs 1 and 8, it seems reasonable to interpret as follows the process which leads to extreme changes like those noted in pig 1. After a few months of fat starvation the fat cells shrink somewhat, but their contents continue to consist wholly of fat. Next the contents of some of the cells break up into larger and smaller fat globules and a material between these globules which is not fat and stains feebly with hematoxylin and basic aniline dyes. This change becomes universal, the cells diminish very much in size, the cell membrane grows irregular in outline owing to the diminution in the volume of the cell body, and the nucleus is no longer applied to the cell membrane but may occupy any position in the cell. In time the much shrunken contents contain no fat whatever and consist merely of a small amount of homogeneous material which stains faintly with hematoxylin, and in some cases of a small amount of finely granular matter near the nucleus. Inasmuch as the transformed layer of adipose tissue maintains almost the volume of an abundant normal subcutaneous fat layer, in spite of the shrinkage of the cells to one-third or one-sixth of their normal size, and inasmuch as there is nowhere any evidence of cell multiplication, it is reasonable to suppose that the volume of
the transformed layer is maintained in part by the presence of a newly formed intercellular substance. Of this the separation of the shrunken cells and the separation of the bundles of elastic fibres into their individual fibres may perhaps be regarded as evidence.

The appearances noted in the epicardial and perirenal fat of pig 8, where, probably owing to more successful preservation, an abundant intercellular substance was present, confirm the opinion here expressed in reference to the nature of the conditions in pig 1.

The histological changes which occur in atrophic adipose tissue have been made the subject of careful study by Flemming,* Czajewicz,† Schmidt‡ and others. Flemming in his classical studies of fat atrophy showed that when fat tissue undergoes atrophy, as seen in experimental starvation or in wasting diseases, the atrophic fat cells do not all undergo the same changes. In many cases three distinct types of atrophy could be distinguished. The commonest form of atrophy is that characterized by a general and evenly distributed reduction in the size of the fat cells, the cells undergoing no alteration either in contour or in the character of their contents. This condition is known as simple fat atrophy. It is often overlooked in the human subject. It is a usual accompaniment of a variety of nutritive and other disorders characterized by wasting.

Another type of fat atrophy is distinguished by cell proliferation. Here the small laterally placed nucleus of the fat cell, together with the small quantity of protoplasm by which it is surrounded, undergoes cell division. As the proliferative process advances the fat content of the cell becomes transformed and disappears. The newly proliferated cells were at one time thought to resemble leucocytes, and Waldeyer maintained that they actually were wandering cells. There are, however, several facts which are inconsistent with this

* Flemming's studies are by far the most important contributions relating to the subject of fat atrophy. The following are his chief writings upon this subject: W. Flemming, Centralbl. f. d. med. Wissenschaft, 1870, No. 31; Arch. f. mikr. Anat., vii (1870), 32 and 328; Virchow's Archiv, lvi (1871), 568 and lvi (1872), 146; Arch. f. mikr. Anat., xii (1876), 391.
‡ H. Schmidt, Virchow's Archiv, cxxviii (1892), 58.
view—the implication of isolated fat cells, the large size attained by
the proliferated cells, their branching form and the manner in which
the cell bodies sometimes remain connected with one another by
slender trabeculae after nuclear division has occurred. This process,
which is especially apt to occur in fat cells where rapid and extreme
wasting has occurred, is described by Flemming as atrophic prolifera-
tion (atrophische Wucherung).

The process of atrophic proliferation differs strikingly from the
third form of atrophy described by Flemming, namely, serous atrophy
(seröse Atrophie) or gelatinous atrophy (gallertige Atrophie) in which
the content of the fat cells is a homogeneous or finely granular
material which contains no fat, and in which the transformed and
atrophied fat cells are separated by a serous or gelatinous substance
which is thought to be of an albuminous nature. This condition of
serous atrophy has been observed by Flemming, Schmidt and others
in atrophic fat in association with the condition of atrophic prolifera-
tion, but it often occurs independently. It has been found in the
human subject in states of prolonged inanition, such as phthisis and
cancer. The alteration is usually confined to the epicardial, perirenal
and omental fat, but is sometimes found elsewhere.

The structural alterations which were observed in pigs 1 and 8
show no definite indications of the process of atrophic proliferation as
it occurs in the atrophy due to the action of iodine, in that which
follows the mechanical constriction of nutrient vessels or in the
atrophy which occurs in rheumatic nodules.* Very few of the
atrophic fat cells contain more than one nucleus, and there is nowhere
seen the cell division which characterizes proliferative atrophy.
Although the intercellular connective tissue is more cellular than that
seen in normal adult fat, this seems to be due partly to arrest of
development, partly to approximation of atrophied cells.

On the other hand, the appearances noted in pigs 1 and 8 bear so

* I have seen a beautiful example of fully developed atrophic proliferation
in a preparation from a small mass of human omental fat which, as the
result of an operation, had suffered constriction from the remaining omen-
tal fat during several days. I am indebted to Dr. Shelby, of the Loomis
Laboratory, for an opportunity to study this material.
close a resemblance to the condition of so-called serous atrophy as it occurs in the human subject that there can be no doubt as to the identity of the processes. The propriety of the name "serous atrophy," implying as it does a replacement of the normal contents of the cell by serum, may well be questioned in view of the observations recorded in this paper. I have, however, continued to use this term as the one generally adopted to designate the same condition in human beings. Comparing the appearances seen in the gelatinous fat atrophy observed in the epicardial fat in wasting diseases, with those which I have produced experimentally, it is found that in both conditions there is a marked shrinkage of all the fat cells, with transformation of their contents, into the same serous or gelatinous material that constitutes the abundant intercellular substance. In both conditions the absence of fat is demonstrable by means of osmic acid. In neither state is the vascularity of the atrophic fat so great as in normal fat. Certain points of difference may, however, be recognized. In the atrophic fat cells of experimental origin only one nucleus is seen in each cell. Very rarely two nuclei, surrounded by a small amount of protoplasm, were demonstrated in the same cell; a larger number was not observed. In preparations made from atrophic pericardial fat, on the contrary, a considerable number of cells appear in which nuclear multiplication has occurred in the atrophic fat cell.

It may be stated, without fuller description of the histology of the proliferative process, that in such cases of serous fat atrophy as have come to my notice in the human subject, the process of atrophic proliferation has co-existed with the process of serous atrophy. In these cases the evidences of cell proliferation have existed in only a small proportion of the atrophic fat cells; the serous atrophy has been the preponderating, but not the exclusive process. In the atrophic fat which develops as the consequence of prolonged fat starvation we may regard the serous form of fat atrophy as the sole lesion. It seems probable, from a consideration of the literature relating to the atrophy of fat, that the experimental serous fat atrophy observed in the animals which form the subject of this paper is the type of serous fat atrophy that has been described.
Fat Starvation and Serous Atrophy of Fat

It seems probable, on experimental grounds, that in pigs at least, serous fat atrophy can be produced only by prolonged deprivation of fat; the removal of carbohydrates, of proteids or of salts giving rise merely to simple atrophy, or to a limited and disseminated serous atrophy. It seems not unlikely that the advanced process of serous fat atrophy observed in the human subject, in the course of chronic tuberculosis and other wasting diseases, depends, similarly, on the presence of a very low percentage of fat in the blood.

Another question which suggests itself is whether the full explanation of the serous atrophy of fat starvation lies in the deprivation of fat to which the organism is subjected. Moreover, does fat starvation under all circumstances entail serous atrophy of fat tissues if the privation be continued long enough? In the case of pig 1 it will be remembered that the supply of proteid and carbohydrate food, though considerable, was restricted. May it not be that simple starvation is in part responsible for the observed changes? The fact that the same kind of changes, though less extreme, was going on in the skin of pig 8 while he was being given an unlimited supply of fatless milk of the same composition as that given pig 1, indicates that general starvation does not play an essential role in producing the beginnings of the condition.

The findings in the case of pig 2 make it clear that the removal of fat from the milk to the extent practised in these experiments does not necessarily induce the serous atrophy. In this animal, as already explained, there was even less fat and proteid ingested than in pigs 1 and 8, but an enormous quantity of carbohydrate food was given. As is well known, mammals have the power of converting carbohydrates into fat, probably chiefly in the liver and through the intermediate stage of glycogen formation, and it is in the highest degree probable that this is the explanation of the absence of the serous atrophy in pig 2. It is to be noted here that though the carbohydrate excess prevented the occurrence of serous atrophy, it was not capable of obviating the gradual absorption and simple atrophy of the subcutaneous fat.

There remains to be noted a collateral feature of considerable in-
terest, namely, the chronic degeneration of the kidney which was observed in pig 1. The explanation of this which appears most probable is that the degeneration resulted from the long-continued excessive activity of the renal epithelium in the excretion of the N-containing constituents of the urine, which, owing to the highly nitrogenous diet, were excreted in pronounced excess. That such exaggerated physiological activity on the part of the renal epithelium may give rise to degeneration was conclusively shown, it is believed for the first time, by the following experiment:

The left or larger kidney was removed from a healthy dog whose daily urea excretion ranged from 10 to 15 grammes on a sufficient diet. When the animal recovered from the operation he was placed on an excessive meat diet, with the result that the excretion of urea was increased to 30 or 40 grammes daily. The remaining kidney was thus compelled to do several times the work which it would ordinarily be required to do. The experiment was continued for one year, during which time the animal was kept in confinement but under otherwise hygienic conditions. He seemed well most of the time, but had occasional vomiting seizures and periods of loss of appetite. He had no symptoms of any acute infectious disease. Autopsy showed that the remaining kidney was perhaps slightly larger than normal; the cortex was rather pale and the markings indistinct, the epithelium of the secreting tubes was the seat of distinct granular degeneration, and there were occasional thin cortical wedges of young connective tissue. For several weeks before the animal was killed the urine had contained a trace of albumin but no casts. The kidney first removed was normal.

Another indication that the changes found in the kidneys of pig 1 were the result of their prolonged overwork is the fact that the kidneys of pig 2, whose nitrogenous food was much more limited than that of pig 1, showed no degenerative change. During the last days of pig 1 there were fever and some pneumatic consolidation, and though it is not likely that the renal changes were the result of infection, its occurrence necessitates caution in the interpretation of the degeneration.
CONCLUSIONS.

The following inferences may be made from the foregoing study:

1. The lesions resulting from fat starvation, at least in the case of pigs, do not resemble or even suggest those of rickets.

2. Prolonged fat starvation leads to the entire disappearance of fat from the adipose tissues. The form of fat atrophy observed as the result of experimental fat starvation corresponds to the serous fat atrophy described by Flemming, and is essentially the same type of fat atrophy as that found in the epicardial and perirenal fat in the human subject as the result of wasting disease.

3. The lecithins of the brain and the fat of the liver are not materially reduced by fat starvation.

4. Fat starvation does not lead to advanced serous fat atrophy of the subcutaneous fat if the animal be given a large excess of carbohydrate food or a considerable excess of the carbohydrate and proteid constituents of milk.

5. Fat starvation causes a very imperfect absorption of the salts of $P_2O_5$ from the intestine.

DESCRIPTION OF PLATES XXIII AND XXIV.

PLATE XXIII.

Shows size and appearance of normal fat cells from subcutaneous fat from back of neck of a normal pig aged one year. Drawn with camera lucida. Ocular 4, objective C (Zeiss).

PLATE XXIV.

Fat from back of neck of Pig 1, one year after commencement of diet consisting of milk from which fat was largely removed. A, fat cells which have undergone serous atrophy. Osmic acid does not stain the contents of these cells. The intercellular matrix is not represented. B, capillaries, some of them with blind ends. C, bundles of elastic fibres. Drawn with camera lucida. Ocular 4, objective C, Zeiss.