CHANGES IN THE BLOOD COMPOSITION OF RABBITS FED ON RAW SOY BEANS.

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(Received for publication, March 29, 1926.)

About 50 years ago Haberlandt (11) wrote that the time would come when soy beans would play a very important rôle in the diet of the poor, and that soy beans would be of much greater importance as admixture for potatoes than salt, because of their high fat and protein content. During the Great War the demand for cheaply produced and easily obtainable nutrients, particularly for suitable proteins and fat, directed attention anew to the possibilities of the soy bean. Daniels and Nichols (9) found that an economic consideration of the yellow soy bean led to the conclusion that it is one of the most valuable of the leguminous seeds. It contains a high percentage of a physiologically good protein, a considerable amount of energy-yielding material in the form of fat and carbohydrate, and a fairly liberal supply of the fat-soluble food accessory, as well as of the water-soluble growth determinant.

Abderhalden (1) found that rats, fed on soy beans, lived from 104 to 315 days. Two of them even increased in weight. Bercxeller (3) also fed rats on raw whole soy bean, soy bean flour, and his patent soy bean flour "O." The rats were able to make a choice of any of these three foods. The results showed a duration of life of 7 to 55 days. The shortest life period corresponded to a high consumption of common soy bean flour, while a higher consumption of raw whole soy bean and of patent soy bean flour O was associated with an increase in the duration of life. The milling process evidently produces a significant change in the grain. Newburgh (24) found that four out of five rabbits after living on soy beans for 2 months showed no changes in the kidneys. But in a period from 4½ to 12 months they regularly acquired chronic renal lesions. These animals had a nitrogen metabolism about twice normal. The blood urea nitrogen showed a rise to 100 mg. per 100 cc. in 7 weeks, while the average value in the normal control period was 31 mg.

Nuzum, Osborne, and Sansum (25) placed a group of rabbits on a 38 per cent soy bean protein diet for a year, in order to study its relation to the production of hypertension. In addition to the soy beans, cod liver oil, tomato, and alfalfa were given. The soy bean group showed an elevation of blood pressure and changes in the blood and urine, while the

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alkaline ash of the soy bean kept the urine alkaline. The carbon dioxide-
combining power of the blood serum remained high. Albumin was not
found regularly in the urine until almost a year after the protein feeding
was commenced, although an increase in the non-protein nitrogen and
urea nitrogen of the blood was noted in the 9th month.

In order to determine the biological value of the soy bean it was
important to know the changes which a diet of soy beans produces
in the chemical composition of the blood.

EXPERIMENTAL.

Seventeen normal rabbits were fed on a preliminary diet of
boiled millet and raw cabbage, their blood taken from the marginal
ear vein, and analyzed for glucose by Folin and Wu’s method, urea
nitrogen by the aeration method, cholesterol by Myers and
Wardell’s method, inorganic phosphorus by Benedict and Theis’
method, and uric acid by Benedict’s method. Later the animals
were put on a diet consisting exclusively of yellow soy beans
soaked in water for 15 hours, and the composition of the blood
determined from time to time. Food was always present in the
cages so that the animals ate more or less constantly. The
results are presented in Tables I, II, III, and IV.

The chemical data show that in general there was an increase
in blood urea, cholesterol, inorganic phosphorus, and uric acid,
following raw soy bean ingestion. The glucose content was
normal or high (minimum 133 mg. per 100 cc.).

Rabbits 5, 8, 9, and 11 died of pneumonia; Rabbits 3, 4, and 6
were killed; Rabbits 1 and 7 died; Rabbits 2, 10, 12, and 15 died
of an unknown cause (nothing abnormal was found at autopsy);
and Rabbits 13, 14, 16, and 17 were still alive at the end of the
experiment. The bodies of the autopsied rabbits showed general
depression of fat. In Rabbits 4 and 6 the gastric mucosa showed
areas of desquamation, indicating an old healed inflammatory
process. A pathological study of the kidneys of Rabbits 1 to 7
was done by Dr. J. R. Cash of the Department of Pathology,
and his findings follow.

Autopsies upon these rabbits do not allow one to draw any definite
conclusions as to the effect of a diet of soy beans upon the kidneys and other
organs. In several of the animals there was almost total macroscopic
disappearance of the body fat but in most cases the rabbits were in a very
good state of nutrition.
**TABLE I.**

*Cholesterol (Whole Blood) in Mg. per 100 Cc.*

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>1</th>
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<th>3</th>
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<th>5</th>
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<th>7</th>
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<td>67</td>
<td>65</td>
<td>52</td>
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<td>43</td>
<td>57</td>
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<tr>
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<td>51</td>
<td>52</td>
<td>62</td>
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<td>77</td>
<td>63</td>
<td>52</td>
<td>75</td>
<td>86</td>
<td>92</td>
<td>67</td>
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<td>74</td>
<td>84</td>
<td>120</td>
<td>87</td>
<td>87</td>
<td>77</td>
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</tr>
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<td>80</td>
<td>49</td>
<td>77</td>
<td>64</td>
<td>50</td>
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<tr>
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<tr>
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<td>63</td>
<td>Died</td>
<td>May 19.</td>
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<td></td>
<td>75</td>
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<td>Died</td>
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TABLE II.
Urea Nitrogen (Whole Blood) in Mg. per 100 Cc.

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<td>Died</td>
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TABLE III.

Inorganic Phosphorus (Blood Serum) Mg. per 100 Cc.

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<td></td>
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<td>Millet and</td>
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</tr>
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<td>5.88</td>
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<td></td>
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</tr>
<tr>
<td>&quot; 13</td>
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<td>5.88</td>
<td>Died</td>
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<td>4.65</td>
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<td>5.00</td>
<td>5.26</td>
<td>5.00</td>
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<td></td>
<td>6.06</td>
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<td>5.00</td>
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<td>5.00</td>
<td>5.40</td>
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<td>soy beans.</td>
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<td>Died</td>
<td>Oct. 26</td>
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</table>
## TABLE IV.

**TABLE IV.**

<table>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric Acid (Blood Serum) Mg. per 100 Cc.</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.16</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3.13</td>
<td>4.00</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>3.16</td>
<td>3.17</td>
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<td>3.66</td>
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<td></td>
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</table>

Food: Raw soaked soy beans.

Millet and cabbage.

Died Oct. 11.

Died Oct. 4.


The kidneys as a rule were swollen and rather pale and in one case (Rabbit 1) were diffusely sprinkled with minute hemorrhages. There were no other macroscopical lesions noted. In no case was there any evidence of arteriosclerotic changes.

Microscopically the kidneys of Rabbit 1 showed many convoluted tubules filled with fresh red blood cells, older casts composed of blood, blood pigment, and desquamated epithelial cells. The epithelial lining remaining in such tubules showed marked infiltration with neutral fat. Here and there were small groups of convoluted tubules and loops of Henle where much fat was present in the epithelium, and a little coagulated albumin was seen in the lumina. The glomerular pores contained a few red blood cells. There was no evidence of the presence of an inflammatory process of any kind.

In Rabbit 7 the convoluted tubules were swollen but practically entirely intact. In a few of these loops and occasionally in a collecting tubule there were small calcified masses which probably represent epithelium which has sometime previously become necrotic. There was no fat or other acute change of any kind present.

In the remaining rabbits a few small areas of mononuclear infiltration were seen and a few of the glomeruli showed delicate adhesions to the capsule. Aside from these rather minor alterations the kidneys seemed normal in all respects.

DISCUSSION.

The yellow soy bean contains an average of 35 per cent of protein, 18 per cent of fat, 27 per cent of nitrogen-free extract, 5 per cent of fiber, 5 per cent of ash, and 10 per cent of moisture. Osborne and Mendel (26) found the proteins of the soy bean, unlike those of the other leguminous seeds thus far investigated, adequate for promoting normal growth. The soy bean is the only seed known to contain both water-soluble and fat-soluble vitamins. Horneman (14) has recently confirmed these findings.

In the present experiments the rabbits were, therefore, fed on a nearly complete food, especially when we take into consideration the fact that the soy beans were soaked for 15 hours, a sufficient time for the accumulation of the antiscorbutic factor. The diet was deficient only in NaCl and Ca. A demonstration of the NaCl deficiency was given by five rabbits of the present series who on the death of Rabbit 9 were found to have torn open

Berczeller’s (3) findings in connection with the duration of life of animals fed on raw soy beans cannot be compared with these because the soy beans used here were thus soaked.
its back and bladder and to have consumed the urine completely, leaving the rest of the body untouched.

The normal and occasional high figures for blood sugar (the blood was taken from non-fasting animals) show that soy beans are not deficient in glucose-yielding substances.

**Cholesterol.**

Horvath and Chang (15) showed that there is an increase of the lipase content in the blood of rabbits fed on raw soy beans. Blood lipase hydrolyzes not only true fats but also other esters of the fatty acids, including lecithin. According to Bloor (6) the unsaturated fatty acids of blood plasma are found largely in combination with cholesterol. A lipase may, therefore, hydrolyze such cholesterol esters with the liberation of free cholesterol. The latter, if deposited in the walls of the blood vessels, might cause arteriosclerosis. This was one of the problems upon which it was hoped the present experiments would throw light.

In our rabbits the cholesterol content of the blood showed a general tendency to rise and reached in two animals twice the initial value. Examination of the kidneys, however, showed no changes in the walls of the blood vessels which would lend support to any conclusion as to the occurrence of sclerosis.

The increase in cholesterol is probably due, at least in part, to the phytosterols known to be present in soy beans. Some investigators have supposed that beriberi is an intoxication with cholesterol. Lawaczeck (cited by Kodama (17)) found that cholesterol was increased in the blood of pigeons fed on a diet deficient in vitamin B. In our experiments the rabbits received a food very rich in vitamin B, but, nevertheless, the cholesterol content of the blood was not reduced to a normal level. It seems, therefore, probable that vitamin B is not directly connected with the metabolism of cholesterol.

**Urea.**

The high figures for urea nitrogen which were obtained in some of our animals even after 1 week of the soy bean diet and after

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*According to Baumann and Holly (2) the average cholesterol content of normal rabbit blood is 67 mg. per 100 cc.*
2 weeks in six rabbits out of seven, can hardly be attributed to the high protein content of the soy bean, because in the experiments of Nuzum, Osborne, and Sansum (25) on rabbits placed on a diet containing 38 per cent soy bean protein, the urea nitrogen did not rise significantly above the average value during the control period (26 mg.) for 3 months and in a year reached a maximum of only 48 mg. On the other hand the microscopic examination gives no reason to believe the high urea content was due to pathological changes in the kidneys of Rabbits 3, 4, 5, and 6, fed on soy beans for 5 weeks. Newburgh (24) reports that in a similar experiment four out of five rabbits showed no changes in the kidneys at the end of 2 months, although the blood urea nitrogen increased to as much as 100 mg. in 7 weeks. We have, therefore, to seek in our case for another explanation. It seems possible that the increase of urea is associated with the high lecithin content of the soy bean, amounting to 1.64 per cent. Marie (22) found in rabbits that subcutaneous injections of lecithin and cholesterol were followed by a four- to sixfold rise in the concentration of blood urea. This may be the explanation of certain cases of high urea in the blood of Oriental people using soy beans as food.

However, another factor must be considered, namely soy bean urease. Carnot and Gerard (8) injected urease into dogs and obtained a disappearance of urea from the blood and a clinical picture of ammonia intoxication, confirmed by the determination of the blood ammonia. At first glance one might expect a drop in the blood urea of rabbits fed on raw soy beans, if the urease penetrates the blood; or, at least, an antagonistic action toward lecithin. Luck (20) found that urease exists in the animal body and recently showed its presence in the gastric mucosa of dogs. If we suppose the soy bean urease to exert its activity in the stomach, where the acidity is very favorable, we may expect penetration of the ammonia into the blood. It would reach the liver and other organs where ammonia is converted into urea. In case a part of the soy bean urease is absorbed by the capillaries of the intestinal tract (as was shown by Horvath and Chang (15) to be the case with soy bean lipase) and carried to the liver, it may aid in the conversion of ammonia into urea, since soy bean urease causes both the synthesis and the decomposition of urea (16, 21).

The presence in the soy bean of another enzyme, uricase (23),
which decomposes uric acid with the formation of urea, may also contribute to the increase in urea.\(^3\)

**Phosphates.**

The inorganic phosphates increased in the majority of the cases. The soy bean contains as mentioned above 1.64 per cent of lecithin, and also a lipase capable of hydrolyzing lecithin. According to Koizumi (18) the introduction of lecithin into the blood results in a distinct increase in the inorganic phosphorus content. Rabbits 8, 9, 10, and 11 were young animals and while fed on millet and cabbage, the inorganic phosphorus content of their blood serum gradually decreased with age, as also occurs in children. Nevertheless, these rabbits gave a marked response to the soy bean food after a week, amounting in Rabbits 8 and 9 to 10.0 and 9.1 mg. per 100 cc. of blood serum, suggesting that the young organism is not able to regulate the inorganic phosphorus level as well as the adult. But in the course of time even the young animals showed a fairly good adjustment to the large amount of phosphorus ingested. Underhill and Bogert (27) showed that rabbits, in contrast to most other herbivora, excrete a considerable amount of phosphates through the kidneys. But they found that after successive subcutaneous injections of phosphate, less of the injected phosphorus was excreted in the urine than after the initial injection.

According to Bloor (5) lecithin probably takes an active part in fat metabolism as the first stage through which the fats pass in their utilization by the organism. The feeding experiments with lecithin performed by Hesse (13) on rabbits confirmed the finding that phosphatides possess a specific action in the deposition of fat. Koizumi (18) found that lecithin, injected intravenously, undergoes decomposition in the body. Its phosphorus in part appears in the blood as inorganic phosphate, and the fatty acids and glycerol unite to form fat. Diets high in fat may, therefore, deprive the body of its phosphorus, which is necessary for the conversion of fat into lecithin. The soy bean, however, is rich

\(^3\) This observation must be taken into consideration in routine clinical laboratories as a possible source of error in the determination of urea nitrogen in blood by the aeration urease method.
in fat and at the same time rich in phosphorus, which is necessary for the metabolism of the former.

Hesse (13) found that vegetable lecithins (phytocithins) are not adequate for the deposition of fat. Berczeller's (3) patent soy bean flour O, containing an average of 20 per cent fat and 0.145 per cent lecithin does not produce obesity in human beings. The bodies of our rabbits fed on soy beans were found practically deprived of fat, but there may be some other explanation for this. In Rabbit 1 the epithelial cells of the tubules in the kidneys were filled with a fatty substance, although in this case the body was also deprived of fat. Newburgh (24) found that the chief deleterious effect of the soy bean diet was exerted upon the epithelium of the convoluted tubules.

Uric Acid.

According to Li Yu Ying (19), the soy bean is poor in nucleoalbuminoids yielding xanthine bases, but contains more paramucleoalbuminoids, which on decomposition do not yield purine bodies but only thymic acid. Some purine bodies are, of course, present in soy beans, but their amount does not seem to have been determined.

The increase in the uric acid content of the blood serum observed in these experiments may be due not only to the presence of purine bodies in the soy beans, but also to the large amount of fat present. Harding, Allin, Eagles, and Van Wyck (12) found that the blood uric acid is raised in human beings on high fat diets, sometimes to 10 mg. per 100 cc. These authors suggested that this increase may be partly accounted for by a decrease in blood volume with a consequent decrease in elimination. It would be simpler to suppose that the absorbed fat, in passing through the stage of lecithin before being utilized, takes the phosphorus from lipoid-like bodies and also from nucleic acid. The nucleins are a possible source of phosphoric acid and at the same time of protein, which may form non-protein nitrogenous bodies. In our results the figures for uric acid probably represent not only uric acid, but also some other closely related substances present in the blood (7). These substances may have originated partly from the soy beans or indirectly from the nucleins of the body, which the soy bean fat separated from their phosphoric acid. Harding, Allin, Eagles,
and Van Wyck (12) noted that on a high fat diet an increase sometimes occurs in the non-protein nitrogen, without any rise in the urea content of the blood. Recently Koizumi (18) has shown the formation of a monoaminomonophosphatide in the liver and also in the mucosa of the intestines. If the absorbed fat takes the phosphorus from such substances and the remaining nitrogenous compound is carried into the blood, an increase of the non-protein nitrogen would result. During muscular work it has been shown that there is a constant production of phosphoric and purine bodies. These substances may both arise in part from nuclein. On a diet high in fat, purine bodies may be liberated from nucleins in a form combined with a pentose. Such a purine pentose compound was found by Davis, Newton, and Benedict (10) in normal cow blood. This point of view is supported by the microscopic picture of lymphocytes taken from exudates of the peritoneal cavities of guinea pigs and rabbits, into which fats had been injected intraperitoneally by Bergel (4). These lymphocytes showed the ingested drop of fat to be closely embraced by the horseshoe nucleus. The fat to be metabolized is carried to the vicinity of nucleins, from which the phosphoric acid necessary for its conversion into lecithin is derived.

The presence of uricase in soy beans must also be taken into account, but the increase in uric acid shows that the combined effect of the purine bodies present in the soy bean and the high fat content is not counterbalanced by the uricase which was described by Němec (23) as "very active."

Rabbits 4 and 6, after being fed on raw soaked soy beans for 5 weeks were sacrificed and immediately autopsied. Desquamation of parts of the gastric mucosa was found and signs of a healed inflammatory process seen on gross examination. Horvath and Chang (15) report similar findings on a rabbit (V) fed on raw soaked soy beans for 9 days. Soy beans contain a very active proteolytic ferment. The mucosa of the stomach which is adapted to withstand the digestive effect of pepsin, may be unable to resist the action of a new proteolytic enzyme such as that of the soy bean. An inflammatory process may well result. In the course of time sufficient adaptation may occur and full resistance be established. This is suggested by the findings in Rabbits 4 and 6 in which after 5 weeks a healed process was present.
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SUMMARY.

1. The blood of rabbits fed on raw soaked soy beans as a single food shows an increase in urea, cholesterol, inorganic phosphorus, and uric acid.

2. The possible sources, direct and indirect, of these increases are discussed.

3. Autopsies showed the body fat to be meager. No arteriosclerosis was found.

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CHANGES IN THE BLOOD COMPOSITION OF RABBITS FED ON RAW SOY BEANS
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