These results are entirely at variance with those of Scolosuboff[1] who says that arsenic is generally found greatest in the spinal marrow, then in the brain, next in the liver, and least in the muscles. His figures calculated to 100 grams of tissue are, taking the muscle content as 1,

Spinal marrow	37.3
Brain	36.5
Liver	10.8
Muscle	1.0

They agree, however, in general with those of Ludwig[2] whose results were

Liver	0.0010 %
Kidneys	0.0004 %
Muscle	0.00025%
Brain	0.0002 %

and with Chittenden[3] who found in material which came to him partly dry and partly preserved in alcohol

1471 g. intestines, contents, and alcohol	0.314 grain As_2O_3
1482 g. liver and alcohol	0.218 grain As_2O_3
331 g. lungs, and spleen (moist)	0.172 grain As_2O_3
603 g. stomach and contents, and esophagus	0.158 grain As_2O_3
411 g. heart and alcohol	0.112 grain As_2O_3
175 g. trachea, larynx, and tongue (dry)	0.081 grain As_2O_3
336 g. brain and alcohol	0.075 grain As_2O_3
83 g. kidneys (dry)	0.029 grain As_2O_3
155 g. diaphragm (dry)	0.010 grain As_2O_3

Chittenden's analyses were made on one or more 100 grams or so samples and the amount of arsenic in the whole organ then calculated. Any conclusions as to the distribution of arsenic in the cadaver drawn from the analyses submitted in the present paper are more to be relied upon than those drawn from any of those quoted, since the material was all obtained in good condition from a cadaver of known history. The contention of Chittenden that "the finding of arsenic in the brain is an indication amounting almost to positive proof that the poison was not *post mortem*" would seem to be confirmed by my findings in this case.

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THE DEVELOPMENT OF FAT IN THE BLACK WALNUT. II.

(Juglans Nigra)

BY F. M. MCCLENAHAN.

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The author has called attention[4] to the significant absence of starch, sugar and especially tannin in the ovule of the black walnut at any time

[1] *Bull. soc. chim.*, [2] **24**, 124.

[2] *Med. Jahrbuch.*, 1880.

[3] *Amer. Chem. J.*, **5**, 8.

[4] THIS JOURNAL, 31, 1093.

during its development from a limpid liquid, June 15th, to a compact solid heavily ladened with fat, Aug. 26th. In contrast with the ovule is the seed-coat of the nut, which indicates at all times during its development the presence of tannin in its tissue.

As with the seed-coat so with the remaining portions of the nut. Tannin is present at all times and in large quantities. It was suggested in the former paper that there may be some genetic relation between the tannin and the content of the nut.

A careful study of cross-sections of the seed-coat by micro-chemical tests indicates that the vascular system thereof is clearly divided in its distribution of fat and tannin. Frozen parts of the seed-coat were prepared and cross-sections made with the microtome. Sudan III indicated no presence of fats in the trachiae and little if any in the phloem. However, ferric chloride indicated the presence of tannin in the phloem both in the outer integument and in the sieve cells. Sudan III gave a very pronounced reaction for fats in the xylem. By dipping cross-sections of the seed-coat in egg albumin to fix the location of the constituents and then in hot Fehling's solution it was demonstrated that there is no reducing sugar in the seed-coat. Potassium iodide iodine gave no reaction for starch or such dextrins as would react with that reagent. Apparently the phloem is rich in tannin and the xylem is not. On the other hand the phloem is not rich in fats while the xylem is decidedly rich in fats. In the former paper attention was called to the very noticeable presence of liquid pressure of the fluid endosperm in the early stages of the development of the ovule. It seems reasonable to assume that the turgidity of the seed-coat is due to an osmosis, in the proper interpretation of which the relative positions of the tannin in the phloem and the fat in the xylem may play an important part. Especially is this thought impressed on one when he observes the richness of the fats in the xylem and the utter lack of fats in the phloem and on the other hand the lack of tannin in the xylem and the richness of tannin content in the phloem.

To throw further light on this interesting problem the late Dr. Waldemar Koch suggested that the author repeat his work on the study of the development of the fat in the ovule of the black walnut, but in addition make an exhaustive study of the relation of the metallic elements to the fats as they occur in the developing seed-coat and ovule of the walnut.

The nuts were taken from the vicinity of Maryville College campus and sent by express to Chicago, where the 90% alcoholic samples of the seed-coats and ovules were prepared. The containers were stoppered with corks carefully covered with tin foil and were protected from light by paper wrappings. During the fall and winter of 1911–12 the extraction and analyses were made in the laboratory of Maryville College.

The medium of extraction for the seed-coats was hot 95% alcohol.

The extraction was readily and completely accomplished with little trouble. The alcohol was at a temperature but little below its boiling point and was poured over the samples several times. The residues dried readily and were considered sufficiently extracted.

The medium for extraction of the ovules was at first hot alcohol applied in a modified form of a soxhlet which allowed the samples to come into direct contact with the vapors of the boiling 95% alcohol. The time of these extractions was from ten to fifteen hours. After that the samples were subjected to about ten hours of extraction with absolute ether dried over sodium. The residues of the samples of seed-coats and ovules were uniformly dried to constant weight over steam at 80°. The residues of the seed-coats gave little trouble in drying and constant weight was easily attained. But the residues of the ovules gave some trouble in being quite markedly hygroscopic so that quick weighing was necessary.

For convenience, the various fractions described hereafter may be specified as follows:

F_1 = hot alcohol-hot ether extractives.....Fats and phosphatids.
F_2 = hot alcohol-hot ether extractives.....Water solubles.
F_3 = hot water extractives.....Salts.
F_4 = residue.....Crude fiber and coagulable proteins.

It is clear that the alcohol ether extractions divided the samples into two parts, F_1F_2 and F_3F_4. Since this paper is not interesting itself with the crude fiber and coagulable proteins of the ovules and seed-coats, our attention will be confined to $F_1F_2F_3$. In all cases F_1F_2 were evaporated to near dryness over steam at 80° and then dried to constant weight in an exhausted desiccator over calcium chloride. F_3F_4 were treated in the same manner, except that in the case of the seed-coat the steam drying was found to be sufficient in the first samples and desiccator drying was subsequently left out. In the case of the ovules the F_3F_4 fractions were found to reach the lowest constant weights if weighed immediately after taken from the steam bath. These fractions were hygroscopic and increased in weight even over the calcium chloride in the desiccator. For this reason the weights from the steam bath which were found to be constant when made quickly were taken as the weights of these fractions also. The combined weights of F_1F_2 and the corresponding F_3F_4 were taken as the total.

F_1F_2 in each sample was transferred to a graduated cylinder and emulsified with a definit amount of chloroform and water, slightly acidulated with concentrated hydrochloric acid. After the aqueous mixture was vigorously shaken it was set aside to allow a separation of the chloroform fraction from the aqueous fraction. The emulsifying required varying amounts of chloroform depending on the fat-lipoid content of the sample, but in every case care was taken not to have an excess of chloroform above what was actually necessary to accomplish the result. The whole water-

chloroform mixture was made up to a definit mark on the cylinder. When the separation of the two layers was complete, which required several hours, aliquot portions in duplicate were pipetted from the supernatant aqueous liquid into weighed porcelain dishes and dried to constant weight over steam at 80°. There was little difficulty in this operation and satisfactory results were readily obtained. By calculation from the total aqueous volume the value of F_2 was gotten. By a subtraction of this value from F_1F_2 the value of F_1 was gotten.

In all cases F_1 and F_2 were ashed by the Neumann method, that is with concentrated nitric-sulfuric acid to a clear liquid and then ignition. The ashing was done in Jena-Kjeldahl flasks and final evaporation and ignitions in silica dishes. It was soon discovered that phosphorus was present in the most minute quantities, only, in the various ash samples and it was decided to use the ammonium phosphomolybdate colorimetric method as employed in water analyses, there being no interfering matter present in the ashes. The estimation of the potassium content was accomplished by the titration of the acetic acid permanganate solution of the potassium cobaltinitrite with standard oxalic acid and back titration with standard potassium permanganate.[1]

The phosphorus obtained from F_1 was calculated as lipoid phosphorus and the lipoid content of F_1 was subtracted from the weight of F_1, the difference being taken as true fat.[2]

This paper concerns itself with the relation of potassium, phosphorus, fat and lipoid and not with the other constituents. It is true that calcium is present in the early history of the ovule, but since its prominence is insignificant after the middle of July not so much importance is placed upon its presence as upon that of potassium in the development of the fat.

The following tables will give the percentage prominence of the various constituents of the ovules and seed-coats at certain specified times during the summer of 1911.

Endosperms.		July 24.	July 31.	August 7.	August 14.	August 21.	Sept. 4.
$F_1F_2F_3F_4$	grams	6.4496	15.9230	13.4266	19.9595	14.0477	36.0461
F_1F_2	%	49.264	53.54	50.47	61.72	62.63	60.55
F_3F_4	%	50.736	46.46	49.53	38.27	37.37	39.45
F_1	%	8.704	30.55	36.71	48.30	53.66	56.83
F_2	%	40.56	22.99	13.76	13.42	8.97	3.72
Fats	%	7.703	30.35	36.46	48.05	53.66	56.83
Phosphatides	%	1.001	0.20	0.25	0.25	.....	....
K_1(as K_2O)	%	0.118	0.125	.....	0.175	0.066	0.015
K_2 (as K_2O)	%	3.359	1.950	1.509	0.483	0.245	0.036
K_3(as K_2O)	%	1.505	0.402	0.397	0.246	0.250	0.129
P_1 (as P_2O_5)	%	0.010	0.014	0.012	0.007	.....	....
P_2 (as P_2O_5)	%	0.010	0.008	0.009	0.007	0.007	0.006
P_3 (as P_2O_5)	%	0.076	0.038	0.069	0.061	0.090	0.189

[1] Bowser, *J. Ind. Eng. Chem.*, 1, 791 (1909).

[2] W. Koch, *J. Biol. Chem.*, 3, 159 (1907).

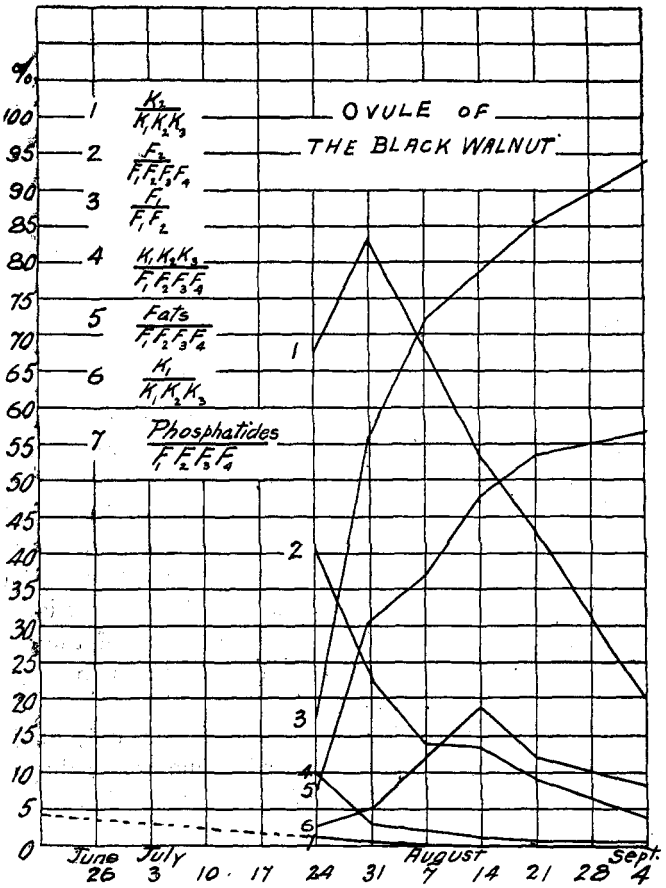
Nucellus.		June 28.	July 24.	July 31.	Aug. 7.	Aug. 14.	Aug. 21.	Sept. 4.
$F_1F_2F_3F_4$	grams	4.4286	4.7489	4.9265	2.1928	1.8310	2.2420	2.4616
F_1F_2	%	31.43	30.52	26.33	26.52	26.40	24.75	37.10
F_3F_4	%	68.57	69.48	73.67	73.48	73.60	75.25	62.90
F_1	%	5.11	17.36	17.68	17.17	15.65	15.83	30.47
F_2	%	26.31	13.16	8.65	9.35	10.75	8.93	6.73
Fats	%	2.43	17.36	17.68	16.64	15.57	15.82	30.29
Phosphatides	%	2.68	.....	.....	0.53	0.08	.....	0.177
K_1 (as K_2O)	%	.....	0.056	0.063	0.061	0.056	0.063	0.042
K_2 (as K_2O)	%	.....	0.329	0.400	0.375	0.129	0.119	0.125
K_3 (as K_2O)	%	.....	.....	.....	.....	.....	.....	.....
P_1 (as P_2O_5)	%	.....	.....	.....	0.038	0.006	.....	0.013
P_2 (as P_2O_5)	%	.....	0.004	0.002	0.030	.....	0.004	0.004
P_3 (as P_2O_5)	%	.....	.....	.....	.....	.....	.....	.....

The fats of the ovules rise from 7.7% on July 24th to 56.83% on Sept. 4th. During the last two weeks of the summer the advance was only 3.17%, showing that the curve of fat development was fast approaching the horizontal. There is no apparent coincidence of development of other constituents of the ovules comparable with the development of the fat content. Nor is there a decline of any important constituent that is comparable with the advance of the fat unless it be the F_2 fraction, that is to say, the water solubles of the ether-alcohol solubles of the ovules. F_1 and F_2 seem to be roughly complementary. F_2 sinks from 40.56% on July 24th to 3.72% on Sept. 4th.

The phosphatides of the ovules were of significance only on July 24th. After that date the F_1 fraction and the fats became practically coincident, due to the low percentage importance of the phosphatides.

TABLE OF RATIOS USED IN THE CURVES.

Ratios of percentages.	July 24.	July 31.	August 7.	August 14.	August 21.	Sept. 4.
$\frac{K_2}{K_1K_2K_3}$	67.42	78.72	....	53.42	43.67	20.00
$\frac{F_2}{F_1F_2F_3F_4}$	40.56	22.99	13.76	13.42	8.97	3.72
$\frac{F_1}{F_1F_2}$	17.67	56.06	72.74	78.25	85.68	93.86
$\frac{K_1K_2K_3}{F_1F_2F_3F_4}$	4.982	2.477	1.906	0.904	0.561	0.180
$\frac{\text{Fats}}{F_1F_2F_3F_4}$	7.703	30.35	36.46	48.05	53.66	56.83
$\frac{K_1}{K_1K_2K_3}$	2.33	5.05	....	19.35	11.76	8.31
$\frac{\text{Phosphatides}}{F_1F_2F_3F_4}$	1.001	0.20	0.25	0.25	....	....



The total potassium content of the ovules represented by $K_1K_2K_3$ dropped gradually from 4.98% on July 24th to 0.180 on Sept. 4th. The relative distribution of K_1, K_2, K_3 may be seen by reference to the accompanying curves. It will be seen that the curves $K_2/K_1K_2K_3$ and $F_2/F_1F_2F_3F_4$ roughly harmonize as to rate of decadence. Also it will be seen that the curves $K_1/K_1K_2K_3$ and $F_1/F_1F_2F_3F_4$ harmonize, roughly during the first half of the summer and during the latter half the former abruptly falls, while the latter tends toward the horizontal. The question quite naturally rises as to the significance of the sudden change in the $K_1/K_1K_2K_3$ curve. It may be pointed out that the tendency toward the horizontal on the part of the fat content is intimately connected with the drop in distribution importance of the potassium that seems to have been associated with the F_1. It may also be true that the increasing relative importance of K_1 during the first three weeks has a vital connection with the very rapid elaboration of fat during those same weeks.

Superficial inspection of the fat collected during the series has shown that the nature of the fat changes during the development of the ovule. The samples collected during the first few weeks became tough and waxy upon continued exposure to air, while the samples of the latter half of the summer tended more and more to remain a permanent liquid. Further work on the study of these samples is planned. The ratio $K_1\%/F_1\%$ may add interest to this point.

July 24.	July 31.	Aug. 7.	Aug. 14.	Aug. 21.	Sept. 4.
1.33	0.403	0.408	0.364	0.123	0.026

This seems to indicate the less and less internal importance of K_1 in the composition of the fats of the ovule and is in harmony with the superficial inspection that the fats tend to become more and more a permanent oily liquid. On the other hand it should be noted that 40.35% fat developed in the ovule during the three weeks in which $K_1/K_1K_2K_3$ was an ascending curve, while only 8.78% fat developed in the ovule during the following three weeks, in which $K_1/K_1K_2K_3$ was a descending curve.

The fats of the seed-coat differ radically. The % presence of the fats in samples, Nos. 2, 3, 4, 5 and 6 remained fairly constant in respect to $F_1F_2F_3F_4$ of the seed-coat, that is to say $F_1/F_1F_2F_3F_4$. It will be noticed that the first sample of the seed-coat was taken June 28th and that sample No. 2 of the seed-coat corresponds to sample No. 1 of the ovule. The ratio $K_1\%/F_1\%$ remained nearly constant in these same samples.

July 24.	July 31.	Aug. 7.	Aug. 14.	Aug. 21.	Sept. 4.
0.323	0.356	0.355	0.357	0.399	0.138

This indicates that K_1 is a constant component in the F_1 of the seed-coat. The phosphatides are of a negligible importance at all times after June 28th and therefore F_1 = fats. By calculating the compound ratio $\frac{K_1\%/F_1\%\ \text{ovule}}{K_1\%/F_1\%\ \text{seed-coat}}$ the following figures are obtained:

July 24.	July 31.	Aug. 7.	Aug. 14.	Aug. 21.	Sept. 4.
4.12	1.15	1.15	1.02	0.31	0.19

It will be seen that at the time in the development of the ovule and seed-coat when $\frac{K_1\%/F_1\%\ \text{ovule}}{K_1\%/F_1\%\ \text{seed-coat}}$ is unity, August 14th, and therefore the potassium of the ovule fat is in equilibrium with the potassium of the seed-coat fat the $K_1/K_1K_2K_3$ has reached its highest point in the potassium distribution of the ovule. This is also the time after which the fats of the ovule tend to remain a permanent oily liquid and after which the accumulation of fats in the ovule decreases rapidly and the curve bends toward the horizontal.

It has been mentioned above that the phosphatides were of negligible importance in the seed-coat after June 28th. On that date they repre-

sented slightly more than 50% of the F_1 of the seed-coat. On the same date the ovule was a limpid liquid, about 3.00% of which represented total solids. About 27% of these solids was the oxides of calcium, potassium and phosphorus. About 3.54% of the total solids was ether extract. (See former paper for these figures.) Although the medium of extraction employed in the former work was ether and not hot alcohol-ether and for that reason probably 3.54% is too low a value for F_1, yet it is scarcely conceivable that the error from this source would amount to more than 50%. Allowing this maximum error, F_1 would have been about 5.00% of the total solids of the ovule or about 0.15% of the whole liquid endosperm. It is impossible at this time to state as to the division of this F_1 between fats and phosphatides, but allowing the curves of fats and of phosphatides to be pushed backward from July 24th to June 28th it will be seen that the fats would be about 1% and the phosphatides would be about 3.5%. On the same date the phosphatides of the seed-coat were 2.68% and the fats were 2.43% of the total solids. It is apparent that this stage in the development of the ovule is conditioned differently from the later stages because of the high percentage content of the salts of calcium, potassium, phosphorus and magnesium present at this time and with the exception of potassium their relative insignificance in the later stages of the development of the nut. The salt of calcium that is most evident is calcium malate.

Summary.

1. The curve of development of fats in the ovule of the black walnut is only roughly complementary to the F_2 of the ovules, which may be seen in the accompanying curve F_1/F_1F_2, representing the following numerical equivalents of the series of ratios:

July 24.	July 31.	Aug. 7.	Aug. 14.	Aug. 21.	Sept. 4.
17.68	57.06	72.73	78.25	85.68	93.86

2. August 14th showed a maximum in the curve of relative distribution, $K_1/K_1K_2K_3$, of the ovules. Before that date the curve ascended, after that date the curve descended.

3. August 14th divides the period of rapid accumulation of fat from the period of slow accumulation of fat in the ovule of the nut. Superficial examination of the several samples indicated that the nature of the fat was not constant throughout the period of development. Before August 14th the fat became waxy on continued exposure to air, after that date it tended to remain a permanent liquid.

4. The curves $F_2/F_1F_2F_3F_4$ and $K_2/K_1K_2K_3$ roughly harmonize throughout the period of study of the ovule, and for that reason it may be concluded that the nature of F_2 does not change materially with respect to its potassium content.

5. The curve K_1/F_1 of the ovule is a descending curve while that of the

seed-coat is roughly constant during the same period of development of the nut. The compound ratio $\frac{K_1/F_1 \text{ ovule}}{K_1/F_1 \text{ seed-coat}}$ becomes unity on August 14th, which fact again makes that date a significant one.

6. The earliest history of the ovule would indicate a great preponderance of phosphatides over fats, which may be noted by an inspection of the backward extension of the phosphatide curve and that of fats in the ovule.

7. The phosphatides linger in the developing ovule until August 14th but their relative importance is insignificant after the fruit has changed from a limpid liquid to a jelly. This is the case with the seed-coat also, except that their importance seems to be nil after the June 28th sample.

8. By a back extension of the potassium curve, $K_1K_2K_3$, it will be seen that the early life of the ovule is conditioned by the presence of a relatively large content of potassium, which becomes less and less important as the fruit advances toward maturity but even on September 4th there is a content equal to 0.18% of the total solids.

9. The nature of the tissue of the seed-coat is such that it is either not penetrable by tannin or contains substances that disrupt the tannin molecule into fragments that under one form or another are able to penetrate the tissue. The line of limitation of tannin penetration in the seed-coat is so clearly marked and yet the premises for a definit conclusion are so fragmentary that this feature of the physiology of the plant life deserves a special study before one should speak finally in reference to the role that fats and tannins play with reference to one another.

The author wishes to express his sorrow on account of the death of the late Dr. Waldemar Koch, whose kindly advice did much to stimulate the effort of this paper, and to thank Dr. William Crocker of the University of Chicago, for his friendly aid and criticism.

MARYVILLE COLLEGE,
MARYVILLE, TENNESSEE.

CORRECTION.

On page 279 of the March number (4th line from the bottom) read *50 mm.* instead of *50 cc.*

On page 280 (last sentence on page) read: The value of K as calculated from its equation, $K = 1/t \log a/a-x$, points, as is seen, to a reaction of the first order with respect to the carbinol base.

NEW BOOKS.

Notes on Chemical Research. By W. P. DREAPER. 68 pp. P. Blakiston's Son & Co.

The work is reprinted from the *Chemical World;* of which the author is editor. In his words, it contains "an account of certain conditions which