The Chemistry of Wheat Germ with Particular Reference to the Scutellum

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In an earlier paper, attention was directed to the surprising concentration of aneurin in the scutellum of wheat (Hinton, 1942). A preliminary figure of 40 i.u./g. was reported as compared with 4 i.u./g. for the next known highest portion of the wheat grain—the embryonic portion of the germ. Ward has also published results which confirm these figures (Ward, 1943).

The present paper reports (a) the results of further work on aneurin distribution in eleven wheat types and four other cereal grains, (b) the results so far obtained in a systematic investigation of the chemistry of the scutellum and embryo of wheat which is being undertaken in these laboratories.

METHODS

Aneurin. The determinations of aneurin were made by the thiochrome method (Nicholls, Booth, Kent-Jones, Amos & Ward, 1942), those on the embryo and scutellum by a micro modification (Hinton, 1943). The embryo and scutellum were dissected from well-formed, whole, dry, individual grains of which about 20 were required to obtain a sufficient sample (5–10 mg.), and the aneurin was extracted by standing overnight at room temperature in 0.2N-HCl.

Other constituents. The rest of the analytical figures were obtained by normal methods applied on a micro-chemical scale, with 10–20 mg. of material dissected as described above, the figures being expressed on an air-dry basis.

N was estimated by Kjeldahl's method; P by Allen's (1940) method; total P, material digested in HNO_3 and H_2SO_4 ; lipid P, material digested in HNO_3 and HClO_4 ; phytate P, material extracted by 0.5N-HCl and doubly precipitated with Ca and Mg (Leva & Rapoport, 1941);* phosphatase, details of the method of estimation will shortly be published by Dr R. G. Booth; lipid, extracted by petrol ether in a reflux type of extractor. The lipid material was dried in a vacuum desiccator at room temperature; no further loss of weight was experienced in subsequent heating at 100° for 1 hr.

RESULTS

(a) Aneurin distribution in wheats and other cereals

The samples of grain were taken from ordinary commercial parcels of unknown history and, with

* Phytate P and total P determinations were carried out by Mr W. J. S. Pringle and it should be mentioned that he has evidence of complication, as yet unsolved, in the determination of phytin in wheat grain. The figures may therefore be amended as a result of further work.

three exceptions, of unknown variety. In some cases figures represent duplicate or triplicate dissections of the embryo and scutellum, in others single dissections (Table 1). From the former it appears that the aneurin figures as a whole may be subject to a sampling variation of about 50 % in the case of the embryo and about 10 % in the scutellum and whole grain. The higher variation in the embryo is probably due to the difficulty of making a perfect dissection free from scutellum rather than to greater variation in its true aneurin content.

The average proportions of the total aneurin contributed by the scutellum (less than 2 % of the grain weight) and the embryo are 59 and 3.1 % respectively. It is interesting to note the high concentration in the scutellum of the hard Durum and Egyptian (which may well be a Durum variety), and the lower concentration in the soft English varieties. The Russian, also high, was a hard red type, very similar in appearance to the Garnet. The figures are, however, too few to be conclusive.

In none of the other cereals examined was the concentration of aneurin in the scutellum as high as in the wheats; in maize, however, the scutellum contributed an even greater proportion of the total aneurin as a result of its large size.

Aneurin content of plumule and radicle portions of wheat. The embryo of Squarehead's Master was divided into two, the radicle and plumule portions. The aneurin contents of the two were:

Plumule (44 % of the embryo)	3.1 i.u./g.
Radicle (56 % of the embryo)	2.4 i.u./g.

Whilst carrying out these estimations it was noted that the water-soluble indicator dye present in wheat germ was confined mainly to the radicle portion. A rapid method for estimating the amount of germ present in cereal products has been put forward, based on the presence of this dye, a water extract being made alkaline and the bright yellow colour produced measured in a colorimeter. This procedure will, however, give reliable results only if the radicle portion is a fixed proportion of the whole germ and this proportion is faithfully represented in the germ material under test.

Table 1. *Aneurin content of scutellum and embryo and their proportion of the grain weight of various wheats and other cereals*

Grains	Average weight of grain (g.)	Aneurin content (i.u./g.) of			Weight (g./100 g. of grain) of		Total aneurin content (% of total in grain) of	
		Grain	Embryo	Scu- tellum	Embryo	Scu- tellum	Embryo	Scu- tellum
		(a) Wheats						
Red Standard	0.055	1.1	1.4	41	1.15	1.74	1.5	65
Squarehead's Master	0.046	1.3	2.7	54	0.97	1.47	2.0	61
White English	0.045	1.3	2.0	53	0.96	1.38	1.5	56
Russian	0.039	1.4	3.1	67	1.03	1.31	2.3	63
No. 1 Garnet	0.034	1.5	4.3	55	1.53	1.78	4.4	65
American Hard Dark Winter	0.026	1.5	7.3	60	1.37	1.37	6.7	55
Egyptian	0.053	1.6	8.1	75	0.92	1.40	4.6	65
No. 1 Manitoba	0.035	1.6	3.1	56	1.30	1.64	2.5	57
No. 4 Manitoba	0.034	1.6	3.9	56	1.38	1.56	3.3	54
Australian	0.048	1.8	4.4	61	1.20	1.54	2.9	52
Durum	0.041	2.0	4.2	70	1.36	1.68	2.8	59
Average				59	1.20	1.54	3.1	59
(b) Other cereals								
Rye	0.032	0.8	2.3	38	1.80	1.73	5.2	82
Barley (husk removed)	0.052	1.1	5.0	35	1.85	1.53	8.4	49
Maize	0.330	1.2	8.7	14	1.15	7.25	8.35	85
Oats (husk removed)	0.038	1.7	4.8	22	1.60	2.13	4.5	28

Aneurin content of germinating wheat. The changes in the aneurin content of scutellum during germination have also been followed (Table 2).

Table 2. *Change in aneurin content of scutellum of wheat during germination*

Days of germination	Condition of seed	Aneurin in scutellum (i.u./g. dry wt.)
1	—	53
2	First root just visible	50
3	First root 1 cm. long	45
5	First root 3-5 cm. long	25

There is obviously an appreciable loss of aneurin from the scutellum when germination is well advanced. Unfortunately, because of the production of substances which seriously interfere with the thiochrome determination, it has not yet been possible to determine the corresponding aneurin values for the growing embryo and the endosperm.

Effect of soaking wheat in water. A sample of Squarehead's Master was soaked in water for different periods, dried, the scutellum dissected and assayed for aneurin, with the results given in Table 3.

Table 3. *Aneurin content of scutellum of wheat after the latter has been soaked in water*

	Aneurin (i.u./g.)
Control wheat	54
After 1 hr. soaking	53
After 3 hr. soaking	47
After 6 hr. soaking	54
After 12 hr. soaking	54

There is no evidence from these figures of a movement of aneurin from the scutellum. This observation is of some theoretical interest, since water enters the wheat grain most readily at the germ end, moving past the scutellum in its path and, further, aneurin is readily extracted from whole commercial germ by the dilute acid (0.2N-HCl) used in preparing the extracts for estimation. The probable explanation for the absence of any transfer of aneurin is that the vitamin is extracted by water only when the cells of the scutellum have been made permeable by the action of acid, chloroform or some similar agent. This is borne out by the figures given in Table 4.

Table 4. *Aneurin extracted from scutellum of wheat under different conditions*

Scutellum:	Aneurin extracted (i.u./g.)
Powdered and extracted by HCl	52
Powdered and extracted by distilled water	31
Powdered and extracted by CHCl ₃ water	51
Large pieces extracted by HCl	62
Large pieces extracted by distilled water	22
Large pieces extracted by CHCl ₃ water	48

Tests showed that the low figures for the distilled water extracts were not due to decomposition of the vitamin, since solutions of aneurin remained stable in distilled water over the period of the experiment. The high figure of 62 i.u./g. for the large pieces of scutellum is in agreement with Ward's figures and suggests that the epithelium half of the

scutellum contains less aneurin than the remainder, since these pieces were cut out, leaving most of the epithelium behind. On another occasion a figure of 67 i.u. was obtained on a similar sample.

(b) *Other chemical characteristics of scutellum and embryo of wheat*

The figures quoted in Table 5 were obtained throughout on a sample of Squarehead's Master.

Table 5. *Chemical characteristics of scutellum and embryo of wheat*

	Total N (%)	Protein N $\times$ 5.27 (%)	Phosphorus				Phytic acid equiv. (%)	Phosphatase (King- Armstrong units)
			Total (%)	Phytate (%)	Non- phytate (%)	Phytate (as % total)		
Scutellum	5.04	28.7	1.90	1.311	0.586	69.1	4.65	58.0
Embryo	5.86	33.4	1.16	0.397	0.760	34.3	1.41	36.0* 56.0†

* Plumule portion of embryo.

† Radicle portion of embryo.

The high protein figure in the scutellum, very little lower than that of the embryo, is not surprising since they are both dense tissues, formed mainly of unvacuolated cells. The higher phosphorus content, 64 % higher than the embryo, is more than accounted for by its greater content of phytate phosphorus.

The author's colleague, Dr R. G. Booth, has carried out determinations of the monophospho-esterase content of scutellum and embryo in dormant wheat on materials provided by the author. The results, incorporated in Table 5, are in general agreement with the findings of Pett (1935) that the scutellum has a greater enzyme content of dipeptidase, protease and lipase than the embryo.

Some constants of the petrol-ether extractable lipid have been determined (Table 6).

Table 6. *Petrol-ether extractable lipid of scutellum and embryo*

	Fat lipid content (%)	Properties of lipid		
		Acid no.	Iodine no. (Wijs)	P content (%)
Scutellum	30.3	5.3	116	0.02
Embryo	15.4	3.7	133	0.03

The petrol ether-extractable lipid from the scutellum is double that from the embryo and is unusually high for a wheat product. The difference in acid number is probably not significant, with regard to the difficulties of measuring it, although in view of the fact that Pett reported approximately four times more lipase in the scutellum of dormant wheat grain than in the embryo, the oil from the former would be expected to have a higher acid number. The lower iodine number of the scutellum lipid suggests that it is of different composition

and it was thought that it might contain a larger proportion of phospholipins which would depress the iodine number. The phosphorus content of the oil from the scutellum, however, is lower than that from the embryo, so this cannot be the explanation. Incidentally, the phosphorus figures are lower than those previously reported for germ oil (see, e.g., Sullivan & Near, 1933), for ethyl ether extraction). The method of estimating the phosphorus was checked on a larger sample of germ oil by a parallel

determination in which the oil was ignited with $Mg(NO_3)_2$ and the P estimated by the Embden-Fetter strychno-molybdate precipitation method. Good agreement between the two methods was obtained.

DISCUSSION

The results presented in this paper extend those previously reported (Hinton, 1942) and show that, in diverse types of cereal grains, the aneurin is concentrated to a greater or lesser extent into the scutellum, this being particularly true of the main commercial types of wheat. This finding may be of importance in the production of bread-making flour of high aneurin content. Thus in a 100 % wheatmeal, using the average figures shown, it can be calculated that the scutellum would account for 0.88 i.u. B₁/g. of flour. If the extraction is decreased, this contribution will increase and in the case of an 85 % extraction flour containing the whole of the scutellum, the latter would contribute 1.03 i.u. aneurin/g. of flour.

The aneurin content of the scutellum of the wheats examined varies from 41 to 75 i.u./g. At the same time, the proportion of the total aneurin of wheats found in the scutellum does not vary much from the average of 59 %, so that the importance of the scutellum is equally great in each case.

The problem of the milling of a high aneurin flour is simply that of the production of a flour containing the maximum amount of scutellum. However, the nature of the practical milling problem thus involved can be gauged by the fact that a variation of 10 % in the scutellum milled into the flour, that is, only 0.15 % of the grist, would produce a variation in aneurin content of the order of 0.1 i.u./g.

Of practical importance is the fact that no loss of aneurin from the scutellum as a result of soaking wheat in water could be detected. Doubt has existed whether the moistening of wheat grain, carried out to adjust suitably the moisture content in preparation for milling, might not produce a redistribution of the aneurin so that the endosperm might gain in the vitamin from the scutellum, which would consequently be of less importance. Such does not seem to be the case; the scutellum will be as important in moistened wheat as in dry. It follows likewise that adjustment of water content as a preparation for milling can be undertaken freely without affecting the validity of any technical development based on the high vitamin content of the scutellum.

A further discussion of the practical aspect of the subject, which should be read in the light of the extended range of figures given in the present paper, has been given by Moran (1942).

There is as yet no indication of the metabolic significance of this concentration of aneurin; no clear conclusions can be drawn from the figures obtained on germinating wheat. There was very little change in aneurin content of the scutellum at 3 days' germination, at which time the dry weight of the embryo had increased three times and erosion of the endosperm was well marked. At 5 days' germination, at which time the aneurin had fallen

by half, the dry weight of the embryo had increased nine times and erosion of the endosperm was well advanced. At this time, the scutellum showed signs of physical change, being dark coloured, horny and brittle, though there was little change in dry weight.

SUMMARY

1. The aneurin content of the scutellum and embryo portion of the germ of various wheat types and of rye, barley, oats and maize has been determined.

2. Expressed as an average for all the wheats, the scutellum was 1.5% of the grain weight and contained 59% of the total aneurin in the grain, whilst the embryo was 1.2% of the grain weight and contained 3% of the total vitamin. Corresponding figures for the other cereals were of the same order.

3. No loss of aneurin from the scutellum could be detected in wheat which had been soaked in water for up to 12 hr.

4. Analytical figures relating to total N, total P, phytate P, phosphatase and petrol ether extract for scutellum and embryo of wheat are given. The scutellum contained 1.3% phytate P, which was more than three times that of the embryo, and 30% lipid material, which was double that of the embryo.

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The Metabolism of Azo Compounds

1. AZOBENZENE

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B. Fischer (1906) described epithelial proliferation following the injection of the azo dyestuff scarlet red. Although the growths were non-malignant he rightly claimed his discovery as the first case of the induction of tumour-like proliferation by a chemical compound. Hayward (1909) found that the active part of the scarlet red molecule was represented by

4'-amino-2:3'-azotoluene, and Yoshida (1934) produced proliferation of liver tissue in mice and liver tumours in rats with this substance. Kinoshita (1937) later examined other derivatives of azobenzene and found *p*-dimethylamino-azobenzene to be highly active in producing liver tumours; and bladder tumours were produced with 2:3'-azotoluene.