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I "SULPHUR DETERMINATIONS IN BRITISH COLUMBIA WHEATS."

II "SELENIUM DETERMINATIONS AND THEIR IMPORTANCE, WITH SPECIAL REFERENCE TO SELENIUM IN BRITISH COLUMBIA WHEATS."

by George Edward Rush

A thesis submitted in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE IN AGRICULTURE in the DEPARTMENT OF AGRONOMY.

The University of British Columbia

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ABSTRACT

I Sulphur and protein were determined on the grain of two varieties of spring wheat, grown at twelve stations in the province of British Columbia. The stations, in representing the principal agricultural areas of the Province, also presented a broad latitudinal array of soil and climatic types. The average sulphur, ranged from a high of .226% at one station to a low of .151% at another station; the protein content ranged from 17.6% to 9.4%. Nowhere were the levels of sulphur so low that support was obtained for the belief that wheat production might be limited by its deficiency. The protein content followed expected trends in response to changes in soil and climate. A highly significant correlation, +.76, was found to exist between total sulphur and protein of the wheat.

II The selenium problem is in review, and a method is outlined for the selenium assay of British Columbia wheat.

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INTRODUCTION:

The agricultural areas in the province of British Columbia, scattered as they are between mountain ranges and located at diverse altitudes, present a broad spectrum of climatic and soil conditions. The agriculturist, therefore, confronted with the problem of determining and recommending crops adapted to the many locations is possessed of a task which is difficult in the extreme. A profitable approach to the problem would seem to lie in studying the responses of genetically similar material of several typical crops grown under widely differing conditions of soil and climate.

Accordingly in 1934, Dr. G.G. Moe, (18) of the Department of Agronomy, of the University of British Columbia, initiated a study with wheat. This crop seemed particularly desirable for the purpose of the study in as much as it was grown to some extent nearly everywhere in the Province, from the extreme north to the American border, in semi-arid areas, and in locations of moderate rainfall. To insure genetic uniformity of the stock, all strains were grown first with careful supervision and selection in the University plots. Seed from these plots was then distributed to selected areas.

The present study arises from the general experiment as a special feature. Information on the distribution in British Columbia of sulphur and selenium, both of which are important in plant and animal nutrition, is entirely lacking. A suspicion exists that sulphur may be deficient in some of the grey wooded soils of the Province and that

this deficiency is expressed in the crops which these soils support. Further, selenium occurs widely in the country rock over extensive areas of the Province, and since it has been reported elsewhere in toxic quantities its distribution in the crops and forages of the Province may be of significance. The wheat samples, grown as they were in all parts of the Province, under diverse soil and climatic conditions offers ideal material for a preliminary study of the distribution of these two elements.

Additional information of scientific value may be obtained from such a study. Since sulphur and selenium occur in the same orbit of the periodic table, a correlative distribution of these two elements would be of interest. Secondly, protein assays were available which made possible correlation studies between the sulphur, selenium, and protein content of the samples.

REVIEW OF LITERATURE:

For more than a century sulphur has been recognized as an indispensable plant nutrient, and for a still longer period fertilizers containing sulphur, for example gypsum (calcium sulphate), have been used both in America and in Europe. Crop responses to these fertilizers however, were attributed to nutrients, other than sulphur contained in them; the early agriculturists, assumed that the natural supply of sulphur was adequate for all crops. It was the Russian chemist Bogdanov (8) who first queried this

assumption in 1899. Several years later three English agronomists put the question to practical field tests (10). Following the work of these pioneers, various localities have been identified in which sulphur is deficient.

Certain American soils, particularly in the northwestern States of Oregon and Washington, are deficient in sulphur (23) (24). When sulphur was applied, marked increases in yields of alfalfa, with slightly less increases for other crops, were evident (23). Newton (20) has shown that the leached, grey wooded soils, or podsols, of Alberta are deficient in sulphur. Spring applications of sulphur or gypsum to clovers at the rate of 100 lbs. per acre produced significant increases in growth. Additional experiments (30) conducted by the Soils Department of the University of Alberta, at Fallis, and at Breton, showed similar results. Unpublished results of experiments conducted by the Department of Agronomy, of the University of British Columbia, on the upland soils of the University farm, showed no increases in yield for red clover. Potatoes, mangels and oats showed approximately a 10% increase. Responses to sulphur have been reported, at least for certain years, in trials with legumes at the Beaverlodge Experimental Station in the Peace River area, and at the Prince George Station in North Central British Columbia (authority verbal).

Usually sulphur is found in the upper layers of a soil and is associated with the organic matter. Soil micro-

organisims transform the organic sulphur to sulphates. These are moderately soluble, and are therefore, subject to movement through the soil. Thus the sulphate content of soils tends to vary inversely with the amount of leaching to which the soil has been subjected.

Sulphur may be added to the soil from the atmosphere by means of rainwater and snow. Amounts of sulphur thus added to the soil varys from a few pounds to fifty or more pounds per acre per year (1). On the other hand, the amount of sulphur lost from the soil due to leaching varies in accordance with the rainfall, but is generally much greater than the amounts added by rainfall and snow (20). The excess of sulphates leached from the soil over the amount added from the atmosphere, and other sources, would continue until after an approximate equilibrium is reached. The soil is left in a condition of relatively low available sulphur.

Owing to the relatively high solubility of most sulphur compounds, it is common to find them leached to depths corresponding to the depth of the percolation water. Areas of concentration of soluble salt will, therefore, often be located at depths equivalent to the extent of the penetration of soil moisture. In humid areas, the sulphur salts generally leach to depths greater than that to which the plant roots penetrate. Whereas in sub-humid and semi-arid regions the concentration zone of soluble salts may

well remain within the feeding range of the plant roots (20). This inverse relationship between leaching and rainfall, together with variations in the sulphur content of parent rock material, and the amount and distribution of organic material in the soil, accounts for the variation of sulphur in soils.

Little work touching on the fundamental aspects of absorption of nutrient elements from the soil has been successful. There is, nevertheless, a large literature dealing with the general aspects, from nutrient solution and fertilizer experiments (6). Little then can be said regarding the uptake of sulphur from the soil other than most physiologists agree that plants absorb sulphur from the soil in the form of sulphates. It is significant to note, however, that while sulphur is absorbed in the form of sulphates, sulphates are not normally found to any appreciable extent in plant tissues, except in the tissues of halophytes. Millar (17) reports that a considerable amount of sulphur found in plants in the form of sulphates results from an excess of the element. This relates to the fact that sulphur taken up by the plant is used largely in the synthesis of protein.

The sulphur compounds found in plants are broadly classified as; (a) sulfoproteins, (b) sulphur containing essential oils and other volatile sulphur compounds, (c) mineral compounds. Cystine, cysteine, and methionine are the major sulphur containing amino acids, and largely account

for the sulphur of protein. Cystine is reported to contain one half of the total sulphur of protein (4). Glutathione, on the other hand, is an important sulphur containing tripeptide probably acting largely as a hydrogen acceptor and taking an active part in cell oxidations and reductions (11). Some of the volatile compounds of sulphur found in plants are mustard oil, allyl sulphide, vinyl sulphide and mercaptans. Mineral forms of sulphur found in plants are generally small and appear to be principally sulphates. Bailey (4) reports the sulphate content of mature wheat plants to be 4-5 percent of the total sulphur.

A few investigators have sought to account for sulphur in intermediary metabolism (28). Most studies, however, deal only with total sulphur (12) and show little attempt to explain its role in the plant cell.

Sulphur deficiency symptoms have been found to be morphologically similar to those of nitrogen deficiency (17). The usual yellowing, or chlorosis, is the most distinctive feature indicated by a deficiency of the element (and nitrogen), and demonstrates the importance of sulphur in chlorophyll formation. Plants deficient in sulphur are also high in carbohydrates and contain more nitrate nitrogen than plants receiving a sufficient supply of sulphur (17). In addition, it has been reported (26) that sulphur deficient plants have longer stems and greater distances between nodes than plants with adequate supplies of sulphur.

An extensive literature relating to sulphur, exists associated with experiments with fertilizer and nutrient solutions. Also, relating chiefly to the lower organisms, a good deal of information exists in respect to sulphur cycles in nature. The sulphur metabolism of the higher animals, too, has been the subject of extensive published investigation. Total sulphur determinations have been made on many higher plants in many places in the world. Beyond, however, demonstrating its wide distribution and its essentiality for growth, science provides only a small fundamental literature relating to the sulphur compounds and sulphur metabolism in the higher plants.

EXPERIMENTAL METHODS:

SAMPLES:

All of the main agricultural areas of British Columbia, with the exception of the Peace River Area, were represented in the wheat trials initiated by Dr. Moe (ibid). Varieties of all the main types of wheat were included in the tests. Field experiments were commenced in 1933 and extended to the end of the 1936 season.

In order to secure genetically uniform seed, the seed for all stations was grown at the University farm under a system which maintained purity of varieties. Each spring or autumn the seed, together with a plan of planting, was sent from the University to the various co-operators. Each variety was grown in triplicate at each station.

Prior to harvesting, properly labelled bags were sent out and heads sufficient for protein determinations were gathered by the co-operators from each of the varietal rows. These samples were forwarded to the University where each variety was checked against the original sample of heads preserved from the previous year. Following threshing and cleaning the samples were sent to the Dominion Grain Research Laboratory at Winnipeg, for protein determinations.

Although both spring and fall varieties were included in the experiment, only spring varieties were used in the sulphur and selenium assays. Two varieties, Marquis and Garnet, were selected as best representing the spring wheats. Both varieties were found to be satisfactory, especially in being free of frost damage at the majority of locations tested. In addition, both Marquis and Garnet were popular varieties at the time the wheat studies were initiated, and were widely grown throughout the spring wheat areas of Canada.

STATIONS:

The twelve stations chosen for the study embrace many types of soil and climate. The wheat grown at these stations becomes an expression of almost as diverse environmental conditions as can be obtained within the geographical latitudes of the Province. The climates vary from mild, coastal types with warm winters and comparatively cool summers at Vancouver, to cold winters and comparatively warm

arid summers at Rock Creek and Kamloops. Colder climates with minimum growing seasons are experienced at stations in north central British Columbia.

Soil types at the stations are represented in azorial soils, highly podsolized glacial tills of the coast, black and brown pedocals of the dry interior, and grey wooded soils of the north central Interior.

The location and characteristics of each station are given, in the brief description that follows, in the map (figure 1), and in Table V.

A. CENTRAL INTERIOR STATIONS

QUESNEL:

Quesnel, is situated on the 53rd parallel, and has a climate more favourable to wheat production than any other northern station tested. The altitude is 1570', with an average frost-free period of 102 days. The silt soils of the alluvial river flats at this station are probably to be classed as grey wooded and somewhat leached. The native vegetation is parkland.

NORTH NEWLANDS AND DEWEY:

These two station are located north-east of Prince George near the northern bend of the Fraser River. The soils are alluvial silts similar to those at Quesnel. Precipitation is relatively high, averaging over 30 inches per year. Coniferous trees predominate throughout the area.

VANDERHOOF:

Vanderhoof is located just north of the 54th parallel on the south bank of the Nechako River. The soils in this area are grey in colour but extremely heavy. Cultivation and preparation of the soil in spring is difficult. The elevation is slightly higher than 2000 feet. Since the frost-free period is only 33 days, damage due to frost is experienced each season. Precipitation is light, supporting a somewhat open mixed forest.

SMITHERS AND QUICK STATION:

Both stations are located in the Bulkley Valley at latitudes 54'50" and 54'45" respectively. The soils in the vicinity of the stations vary considerably but are classified as degraded, black, transition loams. Rainfall is somewhat limited. Winter temperatures are low with an average frost-free period of 54 days. Occurance of frost throughout the region is irregular. Vegetation is predominantly parkland and tall grass.

STREATHAM:

Streatham is located just north of Ootsa Lake at an elevation of 2900'. The soils and vegetation of this area are somewhat similar to those at Smithers and Quick Station. The frost-free period is short because of the high elevation, probably about 80 days.

B. SOUTHERN INTERIOR STATIONS

KAMLOOPS:

Kamloops is situated at the junction of the Thompson and North Thompson rivers, at an elevation of 1159'. The predominating native vegetation is open grassland. The frost-free period for this area is 176 days. In 1934 the plots were located at the base of a hill which subjected the soil to seepage from the adjacent hillside.

The results for 1934 show an unusally low protein content which does not reflect the general behaviour of commercial wheats in the district. The following year the wheat was grown in a place more representative of the district and consequently, the change resulted in a higher protein content in the wheat.

ROCK CREEK:

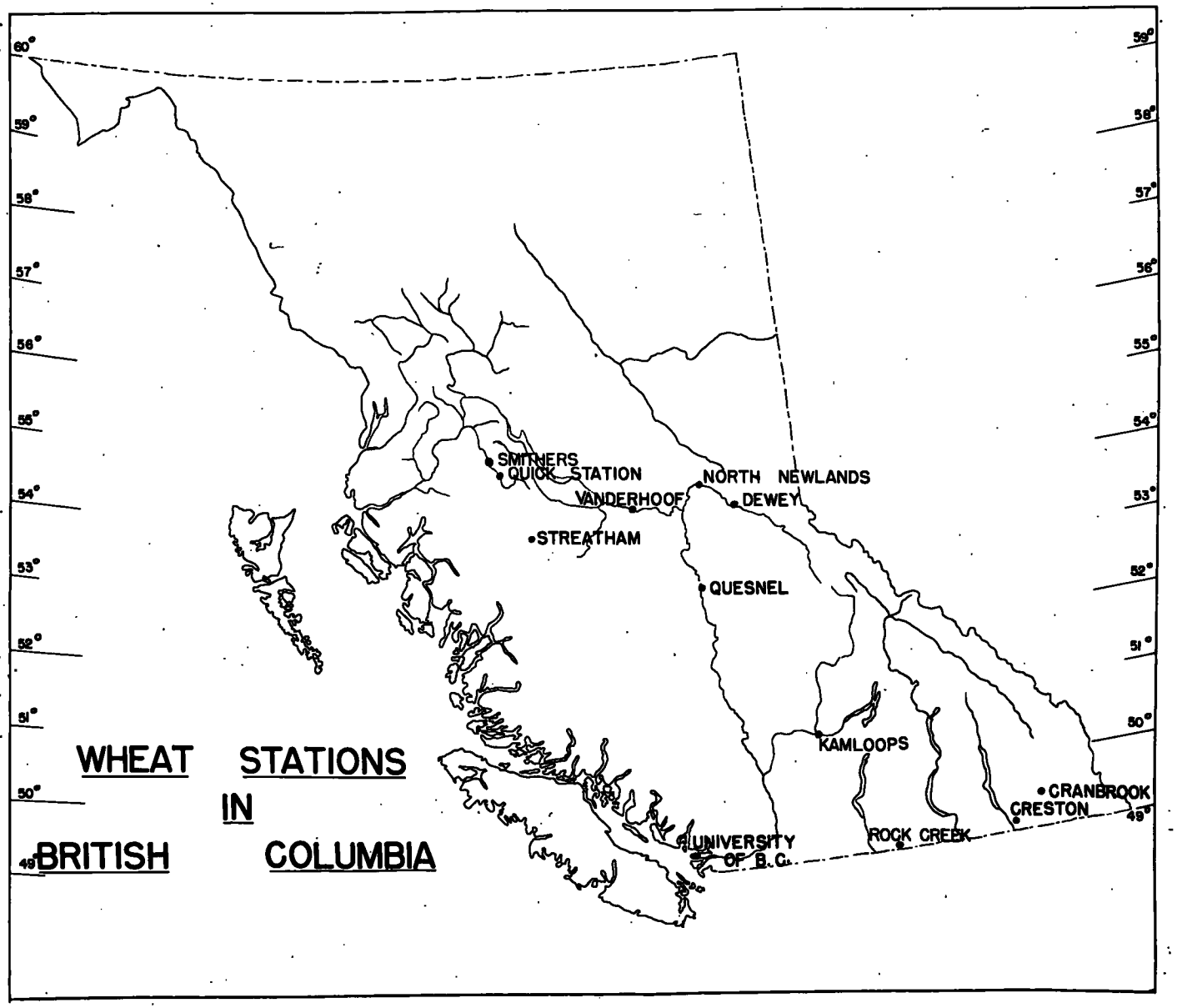
Rock Creek is located south-east of Penticton, and just north of the International Boundary, on low open grassland. The experimental plots were situated at an elevation of 3000', on light, brown soils. The frost-free period for this area is 101 days, the rainfall is limited, and the summer temperatures are high.

C. KOOTENAY

CRESTON:

Creston, is located just north of the International Boundary, on the east side of the Kootenay River. The wheat plots were located at an elevation of 1900', where the

FIGURE 1


WHEAT STATIONS
IN
BRITISH COLUMBIA
SMITHERS
QUICK STATION
VANDERHOOF
STREATHAM
NORTH NEWLANDS
DEWEY
QUESNEL
KAMLOOPS
UNIVERSITY OF B.C.
ROCK CREEK
CRESTON
CRANBROOK
60°
59°
58°
57°
56°
55°
54°
53°
52°
51°
50°
49°

frost-free period is 118 days. The soil has been classed as a fine, silty, alluvium soil. The vegetation of this area is light forest.

CRANBROOK:

Cranbrook is situated north-east of Creston, at latitude 49'31". The wheat plots were located in the district locally referred to as St. Mary's Prairie. The elevation is slightly over 3000'. The frost-free period is 82 days. Precipitation is somewhat limited, but in average seasons it is sufficient for wheat production. The soils of this area are light brown, fine loams, and support an open native grassland.

D. COAST:

THE UNIVERSITY OF BRITISH COLUMBIA:

The University farm is situated at Vancouver, adjacent to the University campus, on glacial upland soil. Climatic conditions are typical of the Pacific Northwest Coast area. The rainfall is considerably more than any of the Interior stations with a lower summer temperature. The virgin vegetation in this area was predominantly heavy, coniferous, forest.

VARIETIES:

MARQUIS:

Marquis, which is a hard, red, spring wheat, was developed at the Dominion Experimental Farms, at Ottawa, and

Agazziz, by Dr. C.E. Saunders. It is a cross between Hard Red Calcutta, and Red Fife varieties. Marquis wheat is an early maturing and relatively high yielding variety, and it is still an important spring wheat in the Canadian northwest.

GARNET:

Garnet, is similar to Marquis, in that it is a hard, red, spring wheat, and was developed at the Central Experimental Farm, Ottawa. It is a cross between two Ottawa varieties namely, Preston A and Rega M. Garnet exhibits somewhat different milling qualities from Marquis. It is not to be compared to Marquis in regard to baking quality. However, Garnet has come to occupy an important place among wheat varieties in those Canadian areas where frost is most feared.

It was felt that the present study would have greater value if these varieties, well adapted to the extreme ranges of climate, and widespread in their use, were chosen for sulphur and selenium determinations.

In some areas damage due to frost, drought, or disease, made it impossible to harvest many of the plots. For these reasons, completely uniform results are not obtained even for Garnet and Marquis, for all years. For sulphur and selenium determinations, triplicate grain samples were bulked and a composite sample drawn for each station and each year in which wheat had been harvested in a normal condition.

ANALYTICAL METHODS:

SULPHUR:

The composite samples were ground in a Wiley mill to pass a 60 mm. mesh, and oven dried at 105°C. for eight hours. A large number of preliminary determinations were made using both the $NaCO_3$ fusion method and the $MgNO_3$ digestion method as outlined in the 1945 edition of A.O.A.C. Methods of Analysis. The latter method was selected after details of the method had been mastered and consistant results had been obtained. The specific procedure adopted consisted of placing approximately 2 grams of oven dry material in a number four porcelain crucible, to which 7.5 ml. of concentrated #$MgNO_3$ was added. The sample was thoroughly mixed and placed on an electric hot plate for five hours. Some difficulty was encountered in preliminary determinations at this stage when it was found that any shorter period of heating failed to complete the digestion. The crucible was then transferred to a muffle furnace, where it was found desirable to raise the temperature gradually to 450°C in order to prevent frothing over of the material. During ignition very voluminous swelling takes place accompanied by crust formation. Great care is required at this stage of the procedure. The temperature was maintained at 450°C. from six

- 37.5 grams of $MgNO_3$ in 250 ml. of H_2O.

to eight hours, depending upon how long it took to drive off the carbon. The digested material was then taken with a 1:1 solution of HCl, filtered, and made up to 250 ml. A 100 ml. aliquot was drawn off and a 10% solution of $BaCl_2$ was used to precipitate the sulphur as $BaSO_4$. Sulphur was determined as o/o of oven dry weight.

EXPERIMENTAL RESULTS:

A. Presentation of data for protein and sulphur.

(1) Protein and sulphur averages for all stations by years and variety expressed as percent dry weight. Tables I, II and III.

(2) Protein and sulphur averages for all stations by geographical areas. Table IV.

(3) Protein and sulphur averages by years only. Table V.

B. Analysis of results for protein and sulphur.

(1) Analysis of variance by years for sulphur. Tables VI, VII and VIII.

(2) Analysis of variance by years for protein. Tables IX, X, and XI.

(3) Correlation analyses: Protein and Sulphur Table XII.

TABLE I

SULPHUR AND PROTEIN MEANS FOR 1934 BY VARIETIES AT 7 STATIONS.

	MARQUIS		GARNET	
STATION	% SULPHUR#	% PROTEIN	% SULPHUR	% PROTEIN
QUESNEL:	.188 ± .001	14.0	.177 ± .000	13.0
NORTH NEWLANDS:	.178 ± .003	11.6	.166 ± .001	10.6
SMITHERS:	.190 ± .099	11.0	.201 ± .000	12.6
KAMLOOPS:	.158 ± .016	9.4	.151 ± .001	9.8
ROCK CREEK:	.161 ± .002	17.3	.162 ± .005	16.4
CRANBROOK:	.213 ± .005	16.9	.216 ± .019	16.5
U.B.C.	.198 ± .098	13.1	.168 ± .065	12.4

- Averages accompanied by their standard errors.

TABLE II

SULPHUR AND PROTEIN MEANS FOR 1935 BY VARIETIES AT 10 STATIONS.

	MARQUIS		GARNET	
STATION	% SULPHUR#	% PROTEIN	% SULPHUR	% PROTEIN
QUESNEL:	.173 ± .000	11.6	.163 ± .000	11.6
DEWEY:	.177 ± .012	13.7	.185 ± .000	12.9
VANDERHOOF:	.208 ± .000	16.5	.183 ± .000	15.6
STREATHAM:	.215 ± .001	11.3	.198 ± .013	11.4
SMITHERS:	.200 ± .000	11.6	.177 ± .000	10.9
QUICK STATION:	.177 ± .002	10.5	.167 ± .022	10.9
KAMLOOPS:	.178 ± .000	12.3	.171 ± .011	11.8
ROCK CREEK:	.167 ± .002	17.5	.172 ± .009	16.6
CRESTON:	.184 ± .008	13.9	.190 ± .013	13.2
CRANBROOK:	.213 ± .011	16.1	.226 ± .000	16.2

- Averages accompanied by their standard errors.

TABLE III

SULPHUR AND PROTEIN MEANS FOR 1936 BY VARIETIES AT 6 STATIONS.

	MARQUIS		GARNET	
STATION	% SULPHUR#	% PROTEIN	% SULPHUR	% PROTEIN
NORTH NEWLANDS:	.159 ± .000	10.3	.161 ± .002	10.0
DEWEY:	.157 ± .000	9.5	.153 ± .002	11.7
VANDERHOOF:	.188 ± .023	15.4	.205 ± .000	14.8
QUICK STATION:	.180 ± .016	10.6	.187 ± .071	10.8
CRESTON:	.180 ± .000	10.8	.186 ± .039	11.0
U.B.C.	.161 ± .002	12.2	- -	11.2

- Averages accompanied by their standard errors.

TABLE IV

PROTEIN AND SULPHUR MEANS FOR ALL STATIONS BY GEOGRAPHICAL AREAS

		MARQUIS		GARNET	
STATION	YEAR	% SULPHUR#	% PROTEIN	% SULPHUR	% PROTEIN
NORTHERN INTERIOR					
QUESNEL:	1934	.188 ± .001	14.0	.177 ± .000	13.0
	1935	.167 ± .000	11.6	.163 ± .000	11.6
N. NEWLANDS:	1934	.178 ± .003	11.6	.166 ± .001	10.6
	1936	.159 ± .000	10.3	.161 ± .002	10.0
DEWEY:	1935	.177 ± .012	13.7	.185 ± .000	12.9
	1936	.157 ± .000	9.5	.153 ± .002	11.7
VANDERHOOF:	1935	.208 ± .000	16.5	.183 ± .000	15.6
	1936	.188 ± .023	15.4	.205 ± .000	14.8
STREATHAM:	1935	.215 ± .001	11.3	.198 ± .013	11.4
	1936	.182 ± .002		.168 ± .001	
SMITHERS:	1934	.190 ± .099	11.0	.201 ± .000	12.6
	1935	.200 ± .000	11.6	.177 ± .000	10.9
QUICK STATION:	1935	.176 ± .002	10.5	.167 ± .022	10.9
	1936	.180 ± .016	10.6	.187 ± .071	10.8
SOUTHERN INTERIOR					
KAMLOOPS:	1934	.158 ± .016	9.4	.151 ± .001	9.8
	1935	.178 ± .000	12.3	.171 ± .011	11.8
ROCK CREEK:	1934	.161 ± .002	17.3	.162 ± .005	16.4
	1935	.167 ± .002	17.5	.172 ± .009	16.6
KOOTENAY					
CRESTON:	1935	.184 ± .008	13.9	.190 ± .013	13.2
	1936	.186 ± .000	10.8	.186 ± .039	11.0
CRANBROOK:	1934	.213 ± .005	16.9	.216 ± .019	16.5
	1935	.213 ± .011	16.1	.226 ± .000	16.2
COAST					
U.B.C.	1934	.197 ± .098	13.1	.168 ± .065	12.4
	1936	.161 ± .002	12.2		11.2

- Averages accompanied by their standard errors.

TABLE V

PROTEIN AND SULPHUR AVERAGES

BY YEARS ONLY.

YEAR	VARIETY	% SULPHUR#		% PROTEIN	
		Mean	Range	Mean	Range
1934	Marquis	.184 ± .007	.055	13.33	7.9
1934	Garnet	.177 ± .008	.065	13.04	6.7
1935	Marquis	.189 ± .008	.048	13.5	7.0
1935	Garnet	.183 ± .006	.063	13.1	5.7
1936	Marquis	.172 ± .052	.031	11.5	5.9
1936	Garnet	.178 ± .071	.052	11.7	4.8

- Averages accompanied by their standard errors.

TABLE VI

AVERAGE SULPHUR AT 7 STATIONS - 1934.
(VARIETIES TWO; DUPLICATE DETERMINATIONS;)

STATIONS	Average sulphur over two varieties % dry wt.
KAMLOOPS	.1548
ROCK CREEK	.1615
NORTH NEWLANDS	.1718
QUESNEL	.1823
U.B.C.	.1830
SMITHERS	.1975
CRANBROOK	.2148

ANALYSIS OF VARIANCE:

Source of Variation	Sum of Squares	D.F.	Mean Squares	Calculated "F"	Tabled "F" @ p = .05
Total	.01276	27			
Stations	.00995	6	.00166	16#	2.92
Varieties	.00030	1	.00030	-	4.6
Stations and Varieties	.00105	6	.00017	-	2.92
Error	.00146	14	.00010		

- Minimum difference between sulphur means required for significance ± .015%

TABLE VII

AVERAGE SULPHUR AT 10 STATIONS - 1935

(VARIETIES TWO; DUPLICATE DETERMINATIONS;)

STATIONS	Average Sulphur over two varieties % dry wt.
ROCK CREEK	.1694
QUESNEL	.1716
QUICK STATION	.1714
KAMLOOPS	.1749
DEWEY	.1824
CRESTON	.1865
SMITHERS	.1888
VANDERHOOF	.1951
STREATHAM	.2065
CRANBROOK	.2201

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Mean Squares	Calculated "F"	Tabled "F" @ p = .05
Total	.01315	39			
Stations	.00992	9	.00110	7.#	2.35
Varieties	.00001	1	.00001	-	
Stations and Varieties	.000207	9	.000023	-	
Error	.00301	20	.00015		

\# - Minimum difference between sulphur means required for significance ± .016

TABLE VIII

AVERAGE SULPHUR AT 6 STATIONS - 1936

(VARIETIES TWO; DUPLICATE DETERMINATIONS)

STATIONS	Average Sulphur over two varieties % dry wt.
DEWEY	.1549
NORTH NEWLANDS	.1601
STREATHAM	.1749
QUICK STATION	.1831
CRESTON	.1862
VANDERHOOF	.1964

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D.F.	Mean Squares	Calculated "F"	Tabled "F" @ p = .05
Total	.00971	23			
Stations	.00505	5	.00101	3.3#	3.11
Varieties	.00001	1	.00001	-	
Stations and Varieties	.00052	5	.00010	-	
Error	.00410	12	.0003		

\# - Minimum difference between sulphur means required for significance ±.024

ANALYSIS OF VARIANCE BY YEARS FOR SULPHUR:

From an analysis of variance by years for sulphur, the following statements can be made:

(1) The sulphur determinations made in the laboratory have been made with acceptable "repeatability". Substantiation of this statement is found in the low values for experimental error given in the several analyses of variance listed in Tables VI, VII and VIII.

(2) The averages are, therefore, acceptably representative of the sulphur content of the given wheat varieties, at given stations, and in given years.

(3) Thus, it follows that different stations may be characterized according to the sulphur content of the wheats grown there. Some stations may be characterized, e.g. Rock Creek, as a "low sulphur" station in 1934, while Cranbrook may be characterized as a "high sulphur" station in the same year. The statistical significance of these observations is established in the highly significant F values "for stations".

(4) Garnet and Marquis wheats do not differ appreciably in sulphur content in any given year. Small differences observed cannot be established as statistically significant (viz. the low F values "for varieties"). One can, therefore, conclude that the provincial average for sulphur for Garnet is not appreciably different from that for Marquis.

(5) Furthermore, Marquis and Garnet, possibly because

of similar heredity, behave with remarkable uniformity irrespective of station, in regard to their sulphur content. When at individual stations, Marquis tends to be high in sulphur, so does Garnet (viz. the low first order interaction of stations and varieties).

(6) While the data by years lacks a certain homogeneity desirable for precise statistical interpretation it can be said in appraisal of all data for all years: that, in spite of marked fluctuations in sulphur in response to season, the relative performance at all stations is remarkably constant. Certain stations, over all seasons, appear to be characteristically "low" in sulphur and certain stations "high" in sulphur.

TABLE IX

AVERAGE PROTEIN AT 7 STATIONS - 1934

(TWO VARIETIES; TRIPLICATE SAMPLES)

STATIONS	Average protein over two varieties % dry wt.
KAMLOOPS	9.6
NORTH NEWLANDS	11.1
QUESNEL	11.6
SMITHERS	11.6
U.B.C.	12.8
CRANBROOK	16.7
ROCK CREEK	16.8

ANALYSIS OF VARIANCE:

Source of Variation	Sum of Squares	D.F.	Mean Squares	Calculated "F"	Tabled "F" @ p = .05
Total	401.2	41			
Stations	280.62	1	46.77	11.6#	2.44
Varieties	.19	6	1.34	-	
Varieties and Stations	8.09	6	1.34	-	
Error	112.30	28	4.01		

Minimum difference between station means to establish significance ±2.4%

TABLE X

AVERAGE PROTEIN AT 10 STATIONS - 1935

(TWO VARIETIES; TRIPLICATE SAMPLES)

STATIONS	Average protein over two varieties % dry wt.
QUICK STATION	10.7
SMITHERS	11.2
STREATHAM	11.3
QUESNEL	11.6
KAMLOOPS	12.0
DEWEY	13.3
CRESTON	13.5
VANDERHOOF	16.0
CRANBROOK	16.1
ROCK CREEK	17.0

ANALYSIS OF VARIANCE:

Source of Variation	Sum of Squares	D.F.	Mean Squares	Calculated "F"	Tabled "F" @ p = .05
Total	315.66	59			
Stations	294.52	9	32.72	80.1#	3.23
Varieties	2.4	1	2.4	6.0	4.07
Varieties and Stations	3.03	9	.33	.8	3.23
Error	15.71	40	.4		

Minimum difference between station means to establish significance ±.76%

TABLE XI

AVERAGE PROTEIN AT 5 STATIONS - 1936

(TWO VARIETIES; TRIPLICATE SAMPLES)

STATIONS	Average protein over two varieties % dry wt.
NORTH NEWLANDS	10.0
DEWEY	10.6
QUICK STATION	10.7
CRESTON	10.9
VANDERHOOF	15.1

ANALYSIS OF VARIANCE:

Source of Variation	Sum of Squares	D.F.	Mean Squares	Calculated "F"	Tabled "F" @ p = .05
Total	115.65	29			
Stations	78.93	4	19.73	13.88	4.94
Varieties	.83	1	.83	-	
Varieties and Stations	7.46	4	1.86	-	
Error	28.43	20	1.42		

Minimum difference between station means to establish significance ± 1.4%

ANALYSIS OF VARIANCE FOR PROTEIN BY YEARS:

From an analysis of variance by years for protein, the following statements can be made:

(1) Variation between replicates for any given variety at a given station, as expressed in protein variability, is small. It can be said, therefore, that the average protein values characterize acceptably both station and variety.

(2) Garnet, and Marquis wheat do not differ from one another appreciably in their protein content under conditions of the experiment (viz. low F values for varieties, Tables IX, X and XI).

(3) Station differences are marked. Some stations, such as North Newlands and Quick, may be characterized as "low protein" stations; other stations, such as Rock Creek and Cranbrook, may be characterized as "high protein" stations. The statistical significance of this is established in the highly significant "F" values for "stations".

(4) The interaction "between stations and varieties" is not significant. Accordingly, it can be stated that Garnet and Marquis, in respect to protein content, tend to behave in a "parallel" manner at all stations. When Garnet is high in protein at a given station, Marquis also tends to be high.

TABLE XII

CORRELATION COEFFICIENTS; PROTEIN AND SULPHUR

Year	Variables	±r
1934	Sulphur to Protein (Marquis)	+.32
1934	Sulphur to Protein (Garnet)	+.40
1935	Sulphur to Protein (Marquis)	+.08
1935	Sulphur to Protein (Garnet)	+.46
1936	Sulphur to Protein (Marquis)	+.55
1936	Sulphur to Protein (Garnet)	+.48
1934-36	Sulphur to Protein (Marquis & Garnet)	+.76
1934-36	Marquis Protein to Garnet Protein	+.60
1934-36	Marquis Sulphur to Garnet Sulphur	+.82

FIGURE 2

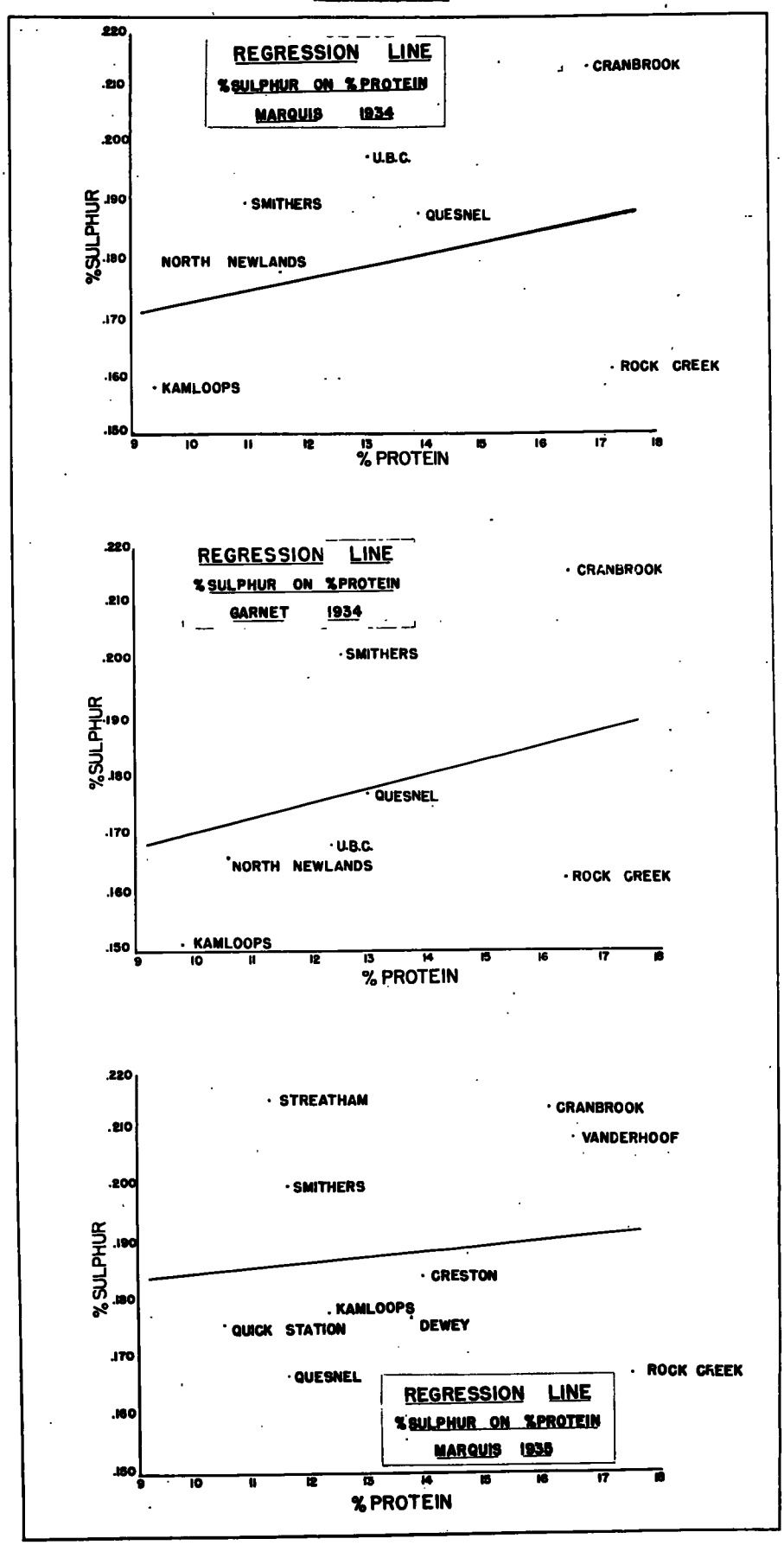

REGRESSION LINE
% SULPHUR ON % PROTEIN
MARQUIS 1934
CRANBROOK
U.B.C.
SMITHERS
QUESNEL
NORTH NEWLANDS
ROCK CREEK
KAMLOOPS
% SULPHUR
% PROTEIN
REGRESSION LINE
% SULPHUR ON % PROTEIN
GARNET 1934
CRANBROOK
SMITHERS
QUESNEL
U.B.C.
NORTH NEWLANDS
ROCK CREEK
KAMLOOPS
% SULPHUR
% PROTEIN
STREATHAM
CRANBROOK
VANDERHOOF
SMITHERS
CRESTON
KAMLOOPS
QUICK STATION
DEWEY
QUESNEL
ROCK CREEK
REGRESSION LINE
% SULPHUR ON % PROTEIN
MARQUIS 1935
% SULPHUR
% PROTEIN

FIGURE 3

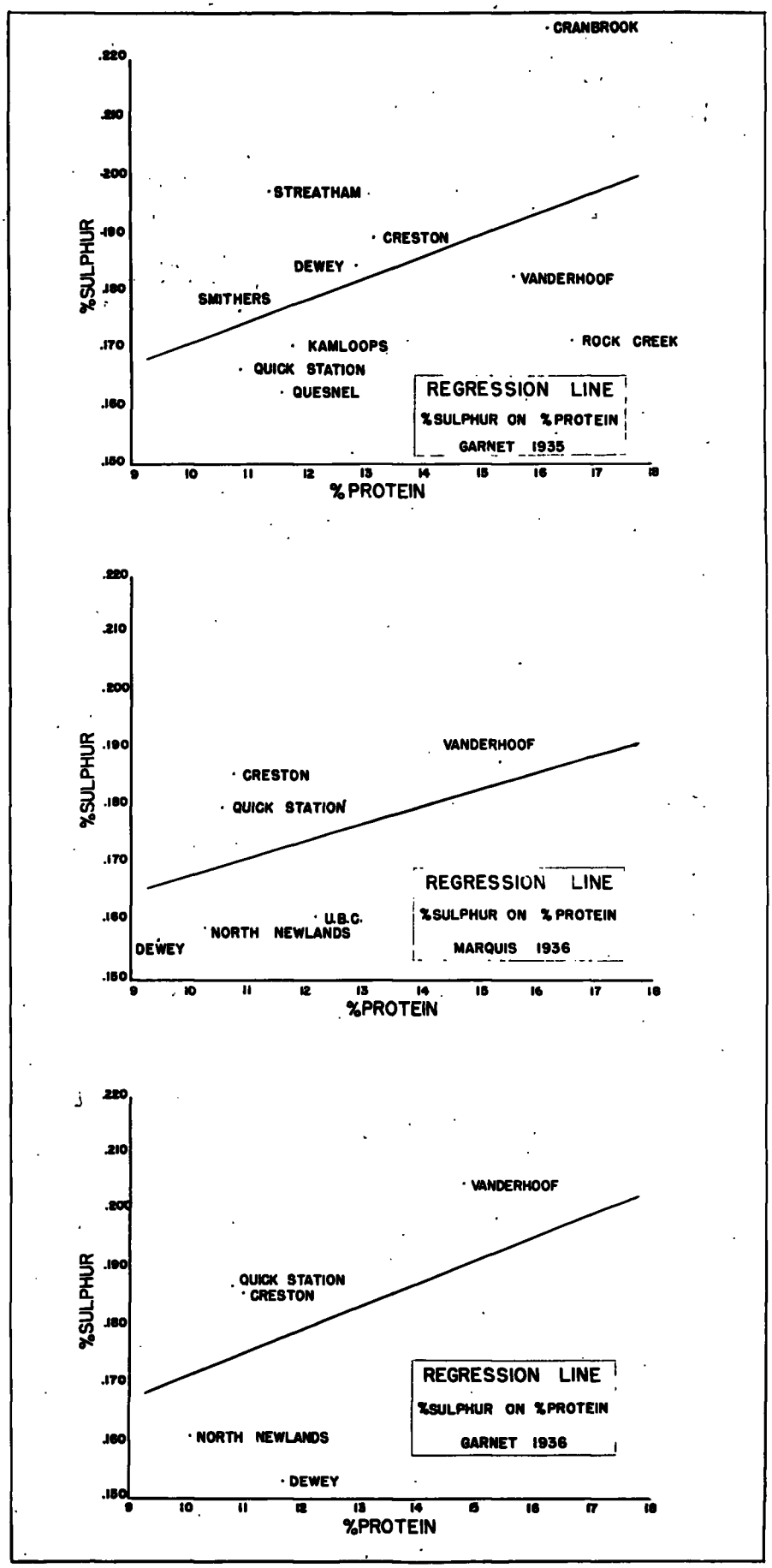
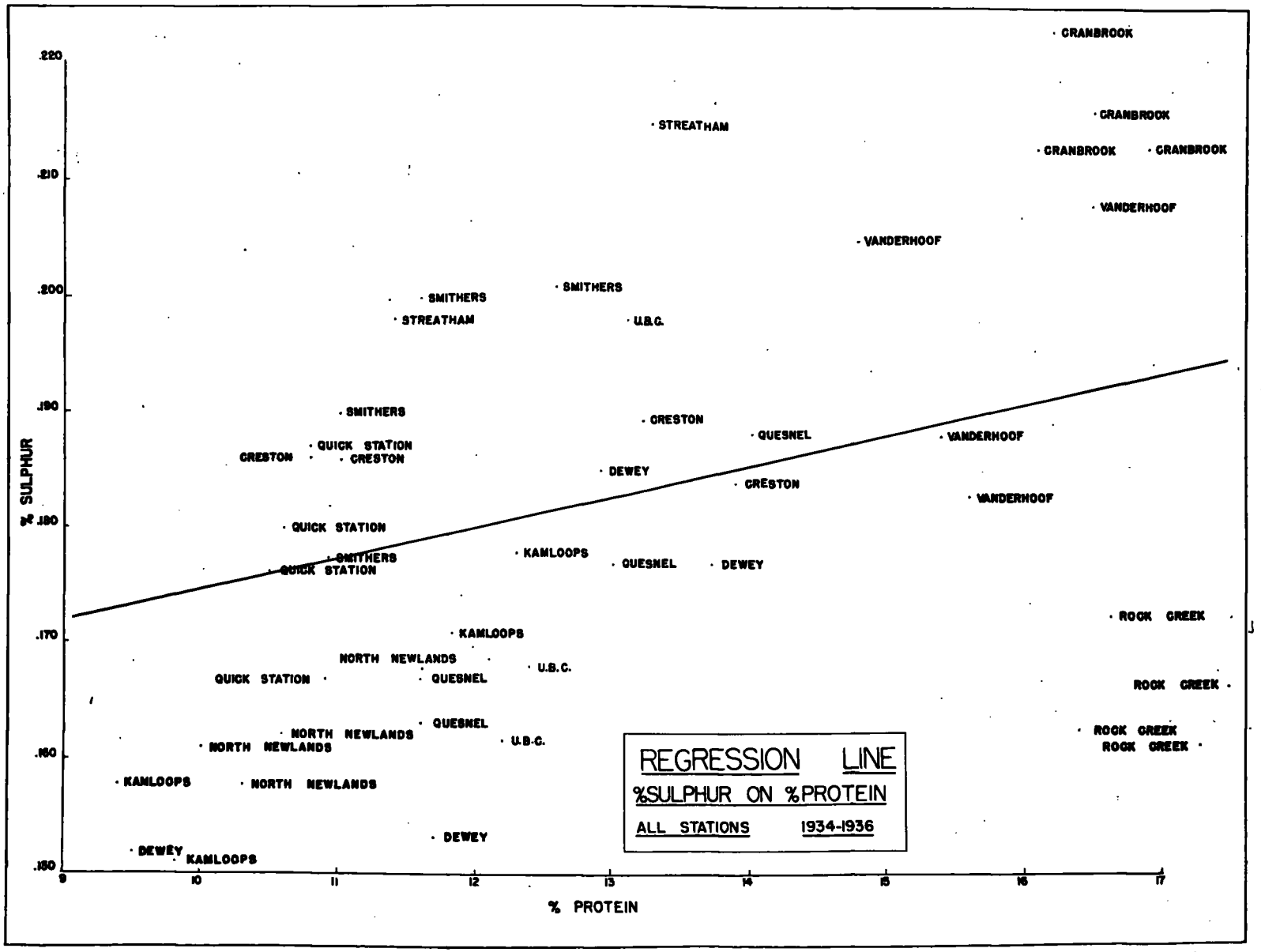

CRANBROOK
STREATHAM
CRESTON
DEWEY
VANDERHOOF
SMITHERS
KAMLOOPS
ROCK CREEK
QUICK STATION
QUESNEL
REGRESSION LINE
%SULPHUR ON %PROTEIN
GARNET 1935
%SULPHUR
%PROTEIN
VANDERHOOF
CRESTON
QUICK STATION
U.B.C.
NORTH NEWLANDS
DEWEY
REGRESSION LINE
%SULPHUR ON %PROTEIN
MARQUIS 1936
%SULPHUR
%PROTEIN
VANDERHOOF
QUICK STATION
CRESTON
NORTH NEWLANDS
DEWEY
REGRESSION LINE
%SULPHUR ON %PROTEIN
GARNET 1936
%SULPHUR
%PROTEIN

FIGURE 4


REGRESSION LINE
%SULPHUR ON %PROTEIN
ALL STATIONS 1934-1936
% SULPHUR
% PROTEIN
.220
.210
.200
.190
.180
.170
.160
.150
9
10
11
12
13
14
15
16
17
CRANBROOK
CRANBROOK
CRANBROOK
CRANBROOK
STREATHAM
VANDERHOOF
VANDERHOOF
SMITHERS
SMITHERS
STREATHAM
U.B.C.
SMITHERS
CRESTON
QUESNEL
VANDERHOOF
QUICK STATION
CRESTON
CRESTON
DEWEY
CRESTON
VANDERHOOF
QUICK STATION
KAMLOOPS
SMITHERS
QUICK STATION
QUESNEL
DEWEY
ROCK CREEK
KAMLOOPS
NORTH NEWLANDS
U.B.C.
QUICK STATION
QUESNEL
ROCK CREEK
QUESNEL
NORTH NEWLANDS
U.B.C.
ROCK CREEK
ROCK CREEK
NORTH NEWLANDS
KAMLOOPS
NORTH NEWLANDS
DEWEY
DEWEY
KAMLOOPS

CORRELATION ANALYSES; PROTEIN AND SULPHUR:

Correlative studies, based on the data presented in Tables I, II, III, and IV reveal the following positive correlation coefficients:

(1) Between protein and sulphur for each variety each year.

(2) Between protein and sulphur over all years and for both varieties.

(3) Between the sulphur content of the Marquis, and the sulphur content of the Garnet wheats.

(4) Between the protein content of the Marquis wheats and the protein content of the Garnet wheats.

In addition to correlation studies, regression lines have been constructed wherein protein content is taken as the independent variable, and the sulphur content as the dependent variable. Regression lines are drawn for each variey each year (Figures 2 and 3) and for all varieties, irrespective of seasons (Figure 4).

From the above information one may conclude:

(1) In wheat grown in British Columbia, there is in general a close relationship between protein and sulphur; as the protein content increases, or decreases, so also does the sulphur content.

(2) The regression lines, however, point to the fact that the above relationship is a generalization and that the ratio of sulphur to protein may vary widely and sig-

nificantly. Hence, at Cranbrook, both sulphur and protein are "high", while at Rock Creek, protein is "high" but sulphur is "low".

DISCUSSION:

Beeson (6), appraising available assays, gives a maximum value of sulphur for wheat grain of .29%, a minimum value of .10%, and an average value of .18%. The sulphur values for the twelve stations in British Columbia fall well within the limits given by Beeson, the British Columbia maximum being .226%, and the minimum .151%. Rock Creek, in the Southern Interior, is relatively low in sulphur, and it is interesting to note that geographically and climatically this small area is related to areas in the interior of Washington State ( ) known to be deficient in sulphur. This does not mean that sulphur is limiting the growth of wheat in the Rock Creek area, but merely that either through improper cropping, or due to some innate soil deficiency, sulphur is low in this area. Successive cropping of the land to high sulphur requiring crops, such as alfalfa, might bring about a deficient condition.

At various times sulphur deficiency has been, as already mentioned, noted in the grey wooded soils of Alberta and British Columbia. In all these cases the deficiencies seem to directly limit the growth of legumes, and indirectly the growth of cereals. Again it appears that wheat grown on certain grey wooded soils, (Table XIII), probably fairly

TABLE XIII

METEORLOGICAL AND SOIL DATA BY STATIONS.

Stations	Altitude	Latitude	Frost-Free Period 32°F	Frost-Free Period 26°F	Precipitation Eff. Index	Temp. Eff. Index	"Probable" Soil Colour	"Probable" Soil Texture and "Type"
NORTHERN INTERIOR								
QUESNEL:	1750'	53'00"	102 dys.	144 dys.	45	37	Grey	Silt - Alluvial - Azonal
PR. GEORGE AREA#	1867'	53'56"	121	158	59	33	Grey	Silt - Lacustrine - Podsol
TELQWA AREA##	2000'	54'45"	54	125	45	29	Degraded Black	Loam - Glacial Till - Podsol
VANDERHOOF:	2093'	54'02"	33	85	39	29	Grey	Clay - Lacustrine - Podsol
STREATHAM:	2900'	53'49"	41	15	57	26	Degraded Black	Loam - Till - Podsol
SOUTHERN INTERIOR								
KAMLOOPS:	1159'	50'49"	176	221	51	51	Light Brown	Fine Loam - Lacustrine-Pedocal
ROCK CREEK:	1992'	49'04"	101	143	###	###	Light Brown	Silt - Alluvium - Pedocal
KOOTENAY								
CRESTON:	1989'	49'06"	118	173	54	42	Light Brown	Fine Silt - Alluvium - Pedocal
CRANBROOK:	3020'	49'31"	82	148	39	37	Light Brown	Fine Loam - Alluvium - Pedocal
COAST								
U.B.C.	45'	49'16"	229	314	134	54	Light Brown	Sandy Loam - Outwash - Podsol

- Includes North Newlands and Dewey.

- Includes Smithers and Quick Station.

- Data not available.

well leached, in north central British Columbia, notably at Dewey and North Newlands, and possibly Quesnel, are low in sulphur. This condition is probably at best a "border line condition" and as noted previously, responses to sulphur, by high sulphur requiring legumes, might be expected in occasional years in these areas.

The protein content of the wheats grown at the twelve stations follow expected patterns in response to climate and soil change. Wheats grown in north central British Columbia, on grey wooded podsolized soils, are in general, low in protein while wheats grown on the pedocals further south are high in protein.

Wheat, as a self-fertilized crop, shows great genetic uniformity within any given variety. Nevertheless, the uniformity of behaviour of Garnet and Marquis at all stations, as shown by non-significant factor interaction, is unusual. As Garnet responds in sulphur and protein content, so also does Marquis. Letham (14) working with 19 spring wheat varieties grown at five southern British Columbia stations came to similar conclusions. An interesting implication derived from such conclusions, is that, for such crops with uniform heredity, a central plant breeding station might, knowing conditions, predict the performance of new varieties in the various sections of the Province.

As shown by Greaves, and Bracken (12) sulphur content in wheat tends to be highly correlated with protein

content. This is borne out in the present study and is to be expected, since an important fraction of wheat protein contains such sulphur containing amino acids as cystine, and methionine. The relationship is only a general one however, since wheat grown at two high protein stations in British Columbia, namely, Cranbrook and Rock Creek, shows a marked difference in sulphur content. At Cranbrook the sulphur content is high, whereas at Rock Creek it is low. This poses the question as to (i) the constancy of the sulphur fraction in wheat protein and (ii) the forms of sulphur in the wheat plant. Greaves and Bracken (12) also noted similar but less striking results obtained in wheats grown under several different cultural conditions. They could not however, account for the "unbalance" in the sulphate sulphur fraction. The question may well be asked, does the sulphur fraction of wheat protein change? Are there, on the other hand, forms of sulphur in the wheat plant about which we know very little? The latter would seem to be more probable.

SUMMARY:

Sulphur and protein were determined on the grain of two varieties of spring wheat, grown at twelve stations in the province of British Columbia. The stations, in representing the principal agricultural areas of the Province, also presented a broad latitudinal array of soil and climatic types. The average sulphur, ranged from a high of .226% at

one station to a low of .151% at another station; the protein content ranged from 17.6% to 9.4%. Nowhere were the levels of sulphur so low that support was obtained for the belief that wheat production might be limited by its deficiency. The protein content followed expected trends in response to changes in soil and climate. A highly significant correlation, +.76, was found to exist between total sulphur and protein of the wheat.

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SELENIUM DETERMINATIONS AND THEIR IMPORTANCE WITH SPECIAL REFERENCE TO SELENIUM IN BRITISH COLUMBIA WHEATS.

DISCUSSION:

For many years the existence of a disease of animals, peculiar to certain districts of South Dakota and adjacent areas, has been recognized and described as "alkali-disease". The disease was first described in 1856 by a United States Army medical officer stationed at Fort Randall Calvalry camp, which was then in Newbraska Territory, but is now in South Dakota. It is of interest to note that he attributed the disease to the pasture, whereas in later years the disease was thought to be caused by the water of the area which is very saline.

In horses, cattle, and swine alkali disease manifests itself by a depressed growth rate, a loss of hair (particularly in the case of mane and tail of horses), and an abnormal development of hoofs, followed, in severe cases, by the sloughing off of old hoofs (12). The more severeley alkalied animals die or have to be destroyed. In poultry the disease affects hatchability.

The South Dakota Agricultural Experiment station investigated water in the disease areas and found it to be harmless (18). In 1928, Dr. Franke, station chemist at the South Dakota Station, began a series of investigations

which definitely determined the disease to be produced by consumption of grain and other vegetation grown upon definite soil areas (13). He was also able, in a general way, to show the extent of areas in which toxic vegetation grew.

In March 1931, a co-operative study of the "alkali disease" problem was undertaken which involved the Bureaus of Home Economics, Animal Husbandry, Plant Industry, Soils and Chemistry. In the early stage of this cooperative study fluorine, lithium and oxalic acid (8) were considered as possible causes of the disease. In the discussions arising at inter-bureau meetings Dr. H.G. Knight, Chief of the Bureau of Chemistry and Soils suggested that selenium be investigated in the toxic grain from the affected areas. As a result of this Robinson (26) worked out a method for determining selenium in soils and wheat, and reported the selenium content of the toxic grain to be 10-12 p.p.m.

A preliminary survey was made in the summer of 1931 and a number of soil and plant samples were collected and their selenium content determined (12). The selenium content of the grains and grasses was found to be correlated with soils derived from Pierre shale. The disease occurs over the entire area where these soils are developed, but is not evenly distributed.

Working with artificially selenized soils, and sound cultures Hurd-Karrer was able to produce seleniferous vegetation which was used by Nelson and Munsell to produce

toxic effects in rats (21).

In subsequent years important progress was made on methods of analysis for selenium, on its physiological role, both in plants and in animals, and on its distribution. Hurd-Karrer (16) found that selenium toxicity was inhibited by applications of sulphur, either as sulphates or elementary sulphur. A ratio of 10 parts of sulphur to 1 part of selenium inhibited toxicity of fairly high concentrations of selenium. Franke and Painter (14) later proved that selenium was associated with the protein fraction of the toxic grains. Since selenium resembles sulphur in its chemical properties they believe it highly likely that selenium partially replaces sulphur in the cystine and methionine portions of the seleniferous protein molecule.

Nelson, Hurd-Karrer, and Robinson (21) reported that plants absorb selenium from the soil and become highly toxic to animals. Quantities as small as 1 p.p.m. of selenium when added to the soil as sodium selenate, permitted typical growth and maturation of wheat plants, with no visible injury to the plants. However, when the grain or straw from these plants was fed to rats and guinea-pigs, it produced toxic effects characterized by retardation in growth, and death occured in a few weeks. Wheat plants containing 10 p.p.m. of selenium produced fatal injury and in many cases visible lesions on the liver. It has been suggested by Byers (7) that vanadium and molybdenum (28) may contribute to the

toxicity of seleniferous vegetation.

Schneifer (28) investigated the toxic effects on rats ingested sodium selenite incorporated in a stock ration. He concludes that selenium disturbs the oxidation-reduction reactions in which sulphur normally plays an important part. Potter and Elvehjem (24) showed that the rate of oxygen uptake by yeast was reduced to one-fifth when sodium selenite was added to a medium of glucose, mannose, or fructose.

The selenium content of plants grown upon seleniferous soils depends upon several factors (2), only one of which is the total amount of selenium present. The form of the selenium is also important. It appears that in the natural sequence of soil formation from seleniferous rocks, "converter" plants such as Astragalus, Aster, and others possess the faculty of absorbing compounds of selenium which are not available to grasses, including wheat. When these plants die and decay they return to the soil forms of selenium compounds which are available to the grasses. Thus, these "converter" plants play an important role in the selenium problem, and areas which produce toxic crop plants are generally infested with "converter" plants (4).

Trelease and Martin (28) in their review of the selenium problem up to 1936 relate the early experiences with toxic loco weeds and the erronious assumptions concerning the role of alkalis in loco poisoning. Couch (10) has attributed the cause of loco poisoning to an organic

nitrogen compound. After finding appreciable amounts of selenium in "Crazyweed" or "Locoweed", Byers (8) suggests that selenium may be a factor in loco poisoning.

Chemical analyses of "indicator" plants have shown that selenium is widely distributed in the soils of the United States (9) and Canada. Plants such as Astragalus, Aster and Loco Weed (Oxytropis Lambertii), each of which have been found elsewhere to contain toxic amounts of selenium, are commonly found among range plants in British Columbia (6). Since selenium in relatively small amounts is toxic to animals (15), some information on the distribution of this element in British Columbia soils and forage would be of value. Beath et. al. (4) have classified wheat as an "intermediate" indicator crop on the basis of experiments which have shown wheat to absorb more selenium from seleniferous soils than grasses, but less than "strong" indicator plants such as Astragulus. The wheat samples obtained by Dr. Moe (ibid), grown as they were in the major agricultural areas of the Province, offer excellent material with which to conduct a preliminary investigation of the distribution and relative concentration of selenium in the soils of British Columbia.

SELENIUM: METHOD OF ANALYSIS.

A MODIFICATION BY DUDLY & BYERS OF THE PROCEDURE DEVELOPED BY ROBINSON ET AL (25).

Place 10-20 grams of ground material in a 400 to 600 ml. beaker and cover with 150-200 mls. of concentrated HNO_3 (Sp. Gr. 1.42). Allow to stand at room temperature for 2-3 hours and stir vigourously at intervals. Add 50 mls. of H_2O_2 (30% by wt.) and allow the mixture to stand overnight. If frothing occurs on addition of H_2O_2, foaming over may be prevented by vigorous stirring. After standing overnight the mixture is warmed slowly on a steam bath until frothing ceases, after which 50 mls. more of H_2O_2 are added, together with 20 mls. of concentrated H_2SO_4. The mixture is then evaporated to almost complete dryness on a steam bath or hot plate. The cooled, black paste is then treated with 100 mls. of HBr (45% HBr) to which has been added sufficient Br to make the mixture deep yellow in colour. The material is then transfered to a 500 ml. round bottomed distillation flask. Apply heat gently. One or two grams of bromine should distill over in the first few mls. of distillate. If insufficient bromine has been added to produce this quantity of bromine in the distillate more bromine must be added. A slight excess of bromine is not harmful, but too much bromine is to be avoided because of the formation of excess H_2SO_4 later. When the danger of frothing is passed, apply more heat and collect 30-50

mls. of distillate. Make a second and even a third distillation with intervening additions of HBr and Br, unless it is certain from experience that all the selenium is in the first distillate.

Remove the distillate and pass in SO_2 until the yellow colour due to the Br is discharged. Add 0.25 to 0.5 grams of hydroxylamine hydrochloride; stopper the flask loosely and put on a steam bath for one hour; allow to stand overnight, at room temperature. If selenium is present it will appear as a characteristic pink or red precipitate. If much selenium is present it will turn black.

Reprecipitation is necessary to free the precipitate from sensible quantities of impurities.

Collect the precipitated selenium on an asbestos pad in a porcelain crucible, and wash slightly with HBr containing a little hydroxlamine hydrochloride. Dissolve the selenium on the asbestos pad by passing through 10 to 15 mls. of a solution containing 1 part of Br to 10 parts of HBr. If the quantity is small and to be determined colourimetrically, wash into a 25 ml. measuring flask. If the quantity is large and to be determined colourimetrically, wash into a 50 ml. measuring flask from which a suitable aliquot may be drawn for colourimetric comparison. Large quantities of selenium (greater than .1 mg.), may be determined gravimetrically. Add 1 ml. of a solution containing 5% gum arabic and precipitate the selenium by adding SO_2 and hydroxylamine

hydrochloride.

Selenium content may be determined by a photoelectric colourimeter, or by comparison with standard solutions. Standard solutions of known amounts of selenium are prepared in the manner outlined above for reprecipitation. Colour comparisons are most accurate when the concentration of selenium is between 0.01 and 0.1 mg.

REAGENTS:

The following special reagents are required for selenium determinations. All reagents must be free from selenium. Blank determinations should be made on all reagents.

1. HBr (40-48%), capable of being decolourized with SO_2.
2. Bromine.
3. A solution of 1 part of Br to 10 parts of HBr.
4. Hydroxylamine hydrochloride.
5. SO_2 as a compressed gas in a cylinder.
6. 5% solution of gum arabic in H_2O.
7. A suspension of asbestos which will not loose weight when washed with strong HBr.
8. An all glass distillation apparatus with thistle tube into a 500 ml. round bottom distillation flask.

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APPENDIX A

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