slowly, giving a brown colour. The brown spots are stable for several weeks in the desiccator, but disappear within 24 h after removal. The purple colour obtained from the second spraying (5 per cent soluble starch) fades in a few hours.

The protein amino-acids and the common sugars gave no reaction. Neither thioethers nor sulphones gave a positive test. Colour formation with di-*n*-propyl thiosulphinate was consistent with a sulphoxide structure. Dimethyl thiosulphonate, diethyl thiosulphonate and toluene sulphinic acid formed a purple colour immediately (that is, without drying) with about one-tenth the sensitivity of sulphoxides (0·1 μmole/20 μl.). 'Cysteine disulphoxide'[3], which probably has a thiosulphonate structure[4], also reacted without drying, but could be detected only at a concentration of 1 μmole per 20 μl. These results agreed with the known reduction[5] of thiosulphonates with hydriodic acid. A weak positive test was obtained with sinigrin, but the explanation for this reaction is unknown. Strong oxidizing agents such as peroxides, peracids, permanganate and periodate form an immediate purple colour with the sulphoxide reagent. Low concentrations of oxidizing agents react at once or not at all. The test is specific for sulphoxides, since they are the only compounds that give a positive test and do not react immediately.

After chromatography, 0·01 μmole of methylcysteine sulphoxide can be detected on a small chromatogram (18 × 18 cm) and 0·05 μmole on a large chromatogram (56 × 56 cm). The type of solvent used did not affect the sensitivity of the test. However, after chromatography with either phenol/water (8 : 3) or *n*-butanol/acetic acid/water (9 : 1 : 2·5), methionine and methylcysteine spots gave a positive test owing to oxidation during evaporation of the solvent after chromatography. The same effect has not been observed with other thioethers or after the use of basic solvents. To avoid oxidation of methionine and *S*-methylcysteine during chromatography[6], they can be converted to their sulphoxides prior to chromatography[7].

More stable thioethers may be chromatographed as such and then detected as sulphoxides after chromatography by treating the chromatogram in a closed chamber with hydrogen peroxide vapours at room temperature for 30 min, followed by thorough drying.

Subsequent to a sulphoxide test, a ninhydrin reaction can be obtained after decolorization of the spots by placing the chromatogram in an ammonia atmosphere for 1 h. This test has already proved useful in the study of the *in vivo* conversion of *S*-methylcysteine to its sulphoxide in crucifers[8], and in the detection of sulphoxides in plant extracts.

We thank J. F. Carson and F. F. Wong for gifts of dimethyl thiosulphonate, diethyl thiosulphonate and di-*n*-propyl thiosulphinate and A. Kjaer for gifts of glucoiberin and sinigrin. This work was partially supported by funds from the Rockefeller grant for research in plant physiology in the College of Agriculture at Cornell University.

JOHN F. THOMPSON
WILFRED N. ARNOLD
CLAYTON J. MORRIS
U.S. Plant, Soil and Nutrition Laboratory,
Soil and Water Conservation Research Division,
Agricultural Research Service,
U.S. Department of Agriculture and Botany Department,
Cornell University, Ithaca, New York.

[1] Kjaer, A., in *Encyclopedia of Plant Physiology*, edit. by Ruhland, W., **9**, 64 (1958).
[2] Toennies, G., and Kolb, J. J., *J. Biol. Chem.*, **128**, 399 (1939).
[3] Toennies, G., and Lavine, T. F., *J. Biol. Chem.*, **113**, 571 (1936).
[4] Sweetman, B. J., *Nature*, **183**, 744 (1959).
[5] Fries, K., and Schürmann, G., *Ber.*, **47**, 1195 (1914). Fromm, E., *Z. Angew. Chem.*, **24**, 1125 (1911).
[6] Dent, C. E., *Biochem. J.*, **43**, 169 (1948).
[7] Thompson, J. F., and Morris, C. J., *Anal. Chem.*, **31**, 1031 (1959).
[8] Arnold, W. N., and Thompson, J. F., *Biochim. Biophys. Acta*, **57**, 604 (1962).

Influence of 5-Bromosalicylhydroxamic Acid on Serum Cholesterol-level

THIS communication presents the results of clinical experiments on influence of 5-bromosalicylhydroxamic acid (BSH) on serum cholesterol-level. BSH was formerly suggested by one of us as an antitubercular agent[1].

W. Johnson *et al.*[2] have given clinical evidence that BSH is an effective inhibitor of isoniazide inactivation. It is known that this inactivation is the result of acetylation of isoniazide[3]. On the other hand, it is known that acetic acid is a precursor of cholesterol in the rat[4] and the biosynthesis of cholesterol can start from acetate[5].

These two facts led us to examine the activity of BSH on cholesterol-level in blood serum. The substance has been shown to be of low toxicity[1,6,7], and this encouraged us to carry out our experiments directly in clinics.

Twenty patients with high cholesterol blood level (above 200 mg) were given orally 4 g of BSH daily. Within twelve days 9 patients (45 per cent) responded favourably to the treatment, and cholesterol-level was reduced by 8–31 per cent (average 20 per cent) (Table 1).

Table 1

No. of patient	Initial cholesterol-level (mg per cent)	Cholesterol-level after twelve days of treatment (mg per cent)	Reduction of cholesterol-level (per cent)	Cholesterol-level ten days after the treatment (mg per cent)
1	238	174	26·9	212
2	272	193	29·1	220
3	220	193	12·3	—
6	248	212	14·8	212
7	193	138	28·3	—
10	248	171	31·0	264
14	287	264	8·0	287
18	248	217	12·5	230
20	207	172	16·9	241
Average	240 ± 9·9	192 ± 11·9	20·0	238 ± 10·5

Ten days after the end of treatment, the cholesterol-level was again examined in most of the patients and was found to be clearly higher than immediately after treatment. A typical example is given in Fig. 1. This seems to indicate that lowering of cholesterol-level was due to the treatment with BSH.

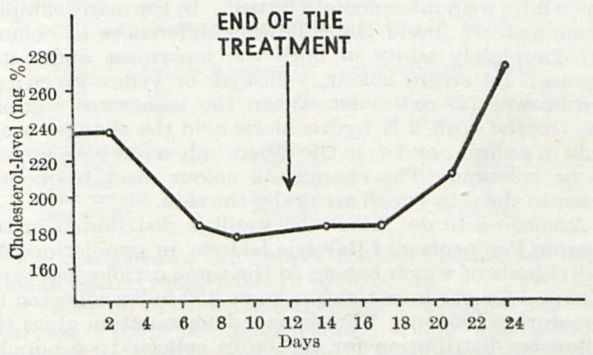

Fig. 1. Influence of BSH (4·0 g daily) on the cholesterol-level in serum

Further experiments will be carried out with the patients which did not respond to the treatment with BSH. In addition to these experiments a number of people with a normal serum cholesterol-level have been examined and their cholesterol, blood sugar and amino-nitrogen in plasma were determined.

The results are summarized in Table 2.

Table 2

No. of persons	Serum cholesterol (mg per cent)			Sugar in blood (mg per cent) (King's method)			Nitrogen of amino groups in plasma (mg per cent) (Frame *et al.* modified by Russel)		
	Hours after the application								
	0	2	4	0	2	4	0	2	4
12	154	167	170	93·5	74·1	78·1	5·1	5·4	5·0
S.E.	±13·3	±14·4	±13·3	±5·1	±2·6	±3·4	±0·17	±0·23	±0·12

It appears that BSH can also slightly raise the serum cholesterol-level in normal persons, and this indicates that

BSH definitely acts on the cholesterol-level, whether it is by reducing or increasing it. The latter seems to characterize cases with a normal cholesterol-level.

BSH certainly reduces the sugar content of blood and it appears that a little increase of amino-nitrogen is possible.

A sedative side-effect of BSH was also noticed in the course of experiments[8].

According to McIsaac and Williams[6] BSH is partly metabolized in the human body into 5-bromosalicylamide. We therefore also examined this substance from the point of view of its activity against hypercholesteræmia. Five patients were treated with 5-bromosalicylamide (2 g daily), but this did not seem to influence the cholesterol-level in blood serum.

A. Czyżyk
T. Urbański

Internal Clinic III,
Medical Academy, Warsaw, and
Institute of Organic Synthesis,
Polish Academy of Sciences,
Warsaw,
Poland.

[1] Urbański, T., Hornung, S., Slopek, S., and Venulet, J., *Nature*, **170**, 753 (1952).
[2] Johnson, W., Mankiewicz, E., Jasmin, R., and Corts, G., *Amer. Rev. Resp. Dis.*, **84**, 872 (1961).
[3] Johnson, W., *Nature*, **174**, 744 (1954).
[4] Bloch, K., and Rittenberg, D., *J. Biol. Chem.*, **155**, 243 (1944).
[5] Bloch, K., *Harvey Lectures*, **48**, 68 (1954); *Vitamins and Hormones*, **15**, 119 (1957). Woodward, R. B., and Bloch, K., *J. Amer. Chem. Soc.*, **75**, 2023 (1955).
[6] McIsaac, W. M., and Williams, R. T., *Biochem. J.*, **66**, 369 (1957).
[7] Hornung, S., *et al.*, *Brit. J. Tuberc.*, **52**, 19 (1958).
[8] Milewski, M. (private communication).

Anthocyanins of the Squill

In the course of an investigation of the cardiotonic glycosides and flavonoid content in the squill (*Scilla maritima* L. or *Urginea maritima* Baker), several anthocyanins were identified, we believe, for the first time.

There are two principal varieties of squill: the red, and the white, with intermediate forms[1]. In the many samples examined, we found the following differences in colour: (*a*) completely white in both the innermost and outer tissues; (*b*) cream colour, yellowish or yellow-green; (*c*) red-brown; (*d*) red-violet. When the transverse sections are treated with 2 N hydrochloric acid the changes occur only in groups *c* and *d*; in the others only a few cells appear to be coloured. The changes in colour most frequently occur in the cells which are under the skin.

According to del Amo[2] the squill is distributed in the Iberian Peninsula and Balearic Islands, in populations the individuals of which belong to the same cariological type. We used for our investigation some 250 bulbs collected by him during 1957–60. Cariological determination gives the following distribution for the bulbs collected: 5 populations towards the north of the Ebro River on the coast of Cataluña which are triploid; 25 populations towards the south of the Ebro on the Mediterranean coast, central part of the Peninsula and Portugal which are all hexaploid; 20 populations collected in the Balearic Islands which are tetraploid. Anthocyanin content has also been examined in other related species: *U. undullata* (Desf.) Steinh., *S. obtusifolia* Moris, *S. lilio-hyacinthus* L., and *S. verna* Huds.

Extractions were carried out by immersing the scales in the presence of absolute ethanol, the final alcoholic concentration being 85 per cent; the dry weight of the bulb was approximately 17 per cent. This was repeated until no more colouring matter could be extracted. No hydrochloric acid was added to the extraction liquids; otherwise the anthocyanins can be altered in part, this being indicated by the appearance of a brown streak near the origin in the chromatogram[3]. A glucoside of pelargonidin which exists in some bulbs was also changed by hydrochloric acid. This does not conform with Lamort's views[4], who maintains that pelargonidin and its glycosides are very stable.

The extracts were concentrated in vacuum and banded on Whatman No. 3 paper, directly or after extracting the major part of the cardiotonic glycosides and flavonoids with ethyl acetate and mixtures of butanol/chloroform[5]. The banding was repeated three times for the isolation of the anthocyanins: first with butanol/2 N hydrochloric acid (1 : 1), afterwards with butanol/acetic acid/water (4 : 1 : 5) and finally with acetic acid/water (15 : 85). The anthocyanins were completely eluted with 70 per cent ethanol. For the identification of the anthocyanins, sugars and acylated residues the general techniques were used[6,7].

The most abundant anthocyanin found in the squill varieties examined was cyanidin-3-monoglucoside, free or acylated with caffeic acid. R_F of the acylated pigment: 0·27, 0·38, 0·42 respectively on the solvents mentioned λ_{max} methanol 0·01 per cent conc. hydrochloric acid = 527 mμ (Lange spectrophotometer II). In some cases other unidentified bands of cyanidin glycosides were found, with lower R_F values when alcoholic solvents were used; some of these glycosides seem to be acylated with *p*-coumaric acid. Two populations of tetraploid plants, examined in August, were found to contain a pelargonidin glycoside, in quantities of about 30–40 times less than those of the cyanidin. It ran in the paper as pelargonidin-3-monoglucoside isolated from the carnation, but was very sensitive to hydrochloric acid. The main difficulty for the identification of some of these pigments is due to the presence of lyophilic substances, which separate well in the chromatograms from the cyanidin-3-monoglucoside, and badly from the other pigments. We are trying to achieve this separation using cellulose powder columns with anhydrous solvents.

Table 1. Anthocyanin Content of Squill Varieties expressed as Chrysanthemin (mg/g Dry Weight)

Polyploidy	Date of determination	No. of populations	Chrysanthemin In dark	Chrysanthemin In light
Triploid	Dec.–Jan.	5	0·00–0·03	1·16–1·76
	May–June	5	0·03–0·18	1·05–1·13
Tetraploid	Dec.–Jan.	8	0·19–1·22	0·57–3·55
	May–June	14	0·06–0·73	0·10–1·60
Hexaploid	Dec.–Jan.	7	0	0
	May–June	12	0	0·00–4·10

The quantitative estimation of the total anthocyanin content of the bulbs was carried out by a colorimetric method (colorimeter Zeiss Elko II, filter *S* 53). The aqueous solutions were made 0·1 N in hydrochloric acid, and the results expressed in cyanidin-3-monoglucoside (chrysanthemin), the same chromatographically isolated pigment used as reference standard. Table 1 records the results of two seasons: in winter when the plants still had leaves, and in late spring when all the leaves were dry. For each population three bulbs of different sizes were examined, immediately after being removed from the earth and after exposing the scales for one month to indirect daylight. When they were exposed to direct sunlight, the anthocyanins were altered after a short time and the scale assumed a brown colour.

Among the bulbs not exposed to light, the greatest content of anthocyanins was present in the tetraploids, which generally are those of greater weight and stronger colour. The triploids contained less and the hexaploids were completely white and contained no appreciable anthocyanins, although small bands of anthocyanins can be seen on the chromatograms. During the seasons mentioned some 60 hexaploid bulbs were examined recently pulled from the earth, only two of which, in the month of May, contained anthocyanins (0·2 mg/g). These results are not given in Table 1 because we have been unable to confirm the cariological determinations.

When the bulbs were exposed to daylight, the anthocyanin content generally increased with the red-violet colour; the white and cream bulbs acquired stronger yellow colour and occasionally yellow-green. From the