1. Hallgren, B. and Larsson, S. *J. Lipid Res.* **3** (1962) 39; **3** (1962) 331.
2. Tsujimoto, M. and Toyama, Y. *Chem. Umschau* 27, 29, 35, 43, (1922).
3. Holman, R. T., Lundberg, W. O. and Malkin, T. *Progress in the chemistry of fats and other lipids* **5** (1958) 118.
4. Brohult, A. *Acta Radiol. Suppl.* **223** (1963).
5. Brohult, J. *Opuscula Medica Suppl.* XII (1969).
6. Brohult, A., Brohult, J. and Brohult, S. *To be published.*

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A General Set of Computer Programs for the Determination of Crystal Structures by Means of Symbolic Addition Methods

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A set of computer programs (GAASA I—VI) for solving crystal structures by "Direct Methods", mainly according to the procedure of symbolic addition, using the Σ_2 relation, developed by Hauptmann and Karle,[1,2] has been written. All space groups, centrosymmetric as well as non-centrosymmetric, are included.

The programs have been written in FORTRAN IV for the IBM-360 system. Each of the six parts is compiled separately, and all data are stored between the different programs on disc or magnetic tape. The core memory requirement is at present 143 000 bytes, which, technically, permits the treatment of structures with 15 000—20 000 reflections in the limiting sphere. For more moderate structures, the core memory requirement could easily be lowered to about 80 000 bytes, but below this limit, considerable rewriting of the programs would be necessary.

The required input is a list of independent, observed structure factors ($h,k,l,|F_o|$, $\sin\theta/\lambda$), preferably stored on disc or magnetic tape. Each of the six subprograms requires, moreover, a number of control cards which define the structural problem.

The course of the phase determination is as follows:

GAASA I — Absolute scale and overall temperature factor according to Wilson[3] are evaluated.

GAASA II — $|E|$ values are calculated from $|F_o|_{abs}$, and $|E|$ statistics are performed. The reflections are sorted in decreasing order of $|E|$.

GAASA III — All symmetry dependent reflections are generated. A list of pairs according to the Σ_2 relation is prepared for each $|E|$ value. Only $|E|$ values greater than a limit, chosen by the user, are taken into account.

GAASA IV — Symbols are assigned for phases or signs of a number of reflections (*e.g.* 10) with large $|E|$ values. Symbolic addition is applied in a cyclic procedure, giving symbols for the phases of new reflections and equations between the symbols, which are subsequently solved. When a sufficient number of symbols have been evaluated, origin determining signs or phases are inserted for the remaining symbols.

GAASA V — Centrosymmetric case. The set of E values are expanded using the Σ_2 summation.

GAASA VI — Non-centrosymmetric case. The phase assignment of the largest $|E|$ values is first performed according to formula *2.9* given by Karle and Karle[2] and the phases are refined using the tangent formula.[4] $|E|_{calc}$ values are then evaluated.

From *GAASA V* or *VI* a list of h, k, l, A, and B ($A=|E|\cos\alpha$, $B=|E|\sin\alpha$) are stored on disc for Fourier summation.

The first tests of this set of programs were performed on two small, already known structures:

(1) Catechol, $C_6H_4(OH)_2$, with one molecule in the asymmetric unit of $P2_1/a$.[5] Signs were evaluated for 109 of 111 reflections with $|E|>1.5$, and all determinations were correct. All heavy atoms were very well resolved in E maps summed with these 109 reflections (11 % of all observed and non-observed reflections), and no additional significant maxima appeared. The execution time for *GAASA I—IV* for this structure is less than 15 min for an IBM 360/50 computer.

(2) Cytosine, $C_4H_5N_3O$, with one molecule in the asymmetric unit of $P2_12_12_1$.[6] 133 reflections (20 %) with $|E|>1.2$ were assigned phases. A comparison with the refined data gave a mean deviation of 14° and a maximum deviation of 64° in α. Most satisfactory E maps with no spurious peaks were also obtained in this case.

Other known structures which have successfully been handled are the ferrocene derivative, $Fe_6C_{70}O_2H_{68}$,[8] and *N*-phenyl-*N'*-benzoylselenourea, $C_6H_5{\cdot}NH{\cdot}CSe{\cdot}NH{\cdot}CO{\cdot}C_6H_5$,[9] with space groups $P\bar{1}$ and $P2_1/c$, respectively. The first unknown structure which was solved with this program set was $K_2CS_3{\cdot}H_2O$.[10]

When coding *GAASA I* and *II*, extensive use was made of *SAP 1* and *2* written by Hall.[11]

The FORTRAN IV listings and a short description of the card input for the program set can be obtained from the authors. A discussion of the methods applied in the programs, and especially in *GAASA IV*, will be published.[7]

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1. Hauptmann, H. and Karle, J. *A.C.A. Monograph No. 3*, The Letter shop, Wilmington 1953.
2. Karle, J. and Karle, I. L. *Acta Cryst.* **21** (1966) 849.
3. Wilson, A. J. C. *Nature* **150** (1942) 151.
4. Karle, J. and Hauptmann, H. *Acta Cryst.* **9** (1956) 635.
5. Brown, C. J. *Acta Cryst.* **21** (1966) 170.
6. Barker, D. L. and Marsh, R. E. *Acta Cryst.* **17** (1964) 1581.
7. Nyborg, J. *Acta Chem. Scand. To be published.*
8. Hanic, F. *Private communication.*
9. Hope, H. *Acta Cryst.* **18** (1965) 259.
10. Philippot, E. and Lindqvist, O. *Acta Cryst. To be published.*
11. *IUCr World List of Crystallographic Computer Programs*, 1966, NRC-4, No. 537.

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Enrichment of Gangliosides in Plasma Membranes of Hamster Kidney Fibroblasts

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Earlier investigations have suggested that animal plasma membranes may contain gangliosides.[1–6] Plasma membranes of hamster kidney fibroblasts (BHK21 cells) have been isolated in this laboratory by using enzyme and antigen markers to follow the isolation steps.[7] These cells do contain gangliosides,[8] and the present report shows that they are enriched in the plasma membrane to a remarkable extent.

Material and methods. BHK21 cells, clone WI-2, were grown as described elsewhere.[9] Fragments of plasma membrane and endoplasmic reticulum were isolated by the method of Wallach and Kamat [10] as described previously.[7] Protein, marker enzymes, and plasma membrane antigens were assayed as described previously.[7] Lipids were extracted from lyophilized samples (2—30 mg protein) by two treatments at 20° with 3 ml of chloroform-methanol (2:1) for 4 h, followed by two similar extractions with chloroform-methanol (1:2). This procedure is believed to extract kidney gangliosides completely.[11] In some experiments the completeness of extraction was actually determined as described by Weinstein *et al.*;[6] only traces of lipids remained in the extracted protein.

To the combined chloroform-methanol extracts was added 6 ml chloroform and 4.5 ml water. The two liquid phases were equilibrated and separated. The lower phase was washed twice with fresh upper phase. The washed lower layer is called the *phospholipid extract.* The combined upper phases were dialyzed against distilled water and lyophilized. The dry residue was extracted 3 times with 3 ml of chloroform-methanol (2:1) and filtered. This solution is called the *ganglioside extract.*

The gangliosides were assayed by the method of Warren.[12] Semiquantitative analyses were carried out also by thin layer chromatography (TLC) on silica gel G plates with propanol-