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Contractor: BIONETICS RESEARCH LABORATORIES, INC.
Contract No.: DA 18-108-AMC-119(A)

SECOND QUARTERLY PROGRESS REPORT

Covering the Period

22 June 1963 - 22 September 1963

Title: New Methods Development for Irritant Screening

Prepared by

E. ROSS HART, Ph.D.

Date: 15 October 1963

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Bionetics Research Laboratories, Inc.
Falls Church, Va.
NEW METHODS DEVELOPMENT FOR IRRITANT SCREENING - E.Ross Hart
Report No. 2, 22 September 63, 5 pp
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4C08-03-016-07

During second quarter, assessment by current methods was emphasized. Screening completed on approximately 100 compounds. Remainder of 100 compounds will be completed by middle of third quarter. Rat mast cells supply inadequate histamine for test of liberation by irritants.

1. Irritation, skin and eye
2. Contract DA 18-108-AMC-119(A)

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INTRODUCTION

This second quarterly progress report presents a statement of work accomplished during the period from 22 June, 1963 to 22 September, 1963 by the contractor, Bionetics Research Laboratories, Inc., under Contract No. DA 18-108-AMC-119(A).

The report is divided into sections which correspond to the sections of the Statement of Work of the Contract. Thus, Section I (New Methods Development) discusses the progress which has been made in that phase of the work. Section II (Assessment of Irritant Potential) discusses the screening operations under the corresponding phase of that work.

It seems appropriate to point out here that, in accordance with plans developed in conference with the contract project officer, the screening work (Section II) has received the larger share of emphasis during the period herewith reported upon. This was done with the objective of achieving a high degree of efficiency of operation by utilizing a well-trained team full time. That this objective has been achieved will, it is believed, be clear from what follows in Section II. In future period the proportion of emphasis on the two phases of the contract will shift, for the obvious reason that the screening work is nearing completion.

SECTION I - New Methods Development

A. Work accomplished

Histamine liberation

As mentioned in the first progress report, the decision was made that the investigation of the histamine-liberating characteristics of irritants was to be explored first. It was further decided that the amounts of histamine present in tissues before and after exposure to irritants would be measured by a fluorometric technique, and that the first "tissue" examined would be mast cells isolated from rat peritoneal fluid. At present the fluorometric assay of histamine is proceeding smoothly and presents no difficulties. The method for collection of mast cells originally adopted (Uvnäs and Thon, Exptl. Cell Res., 18: 512, 1960 and 23: 45, 1961) presented difficulties in that it provides an inadequate number of mast cells. It has, therefore, been necessary to modify this procedure. That is, instead of withdrawing fluid from the peritoneal cavity of animals previously injected and sacrificed, we are now maintaining a number of animals with regular peritoneal injections and fluid withdrawals in order to produce more massive ascites and consequently larger numbers of mast cells. Preliminary evaluation of mast cells as a source shows that only a limited amount of histamine is released on incubation with irritants. Furthermore, it has thus far shown the differences between irritants to be disappointingly small. At present it is felt that a more adequate survey should be made of the various conditions of incubation of irritants with the cells before this technique is abandoned.

Species Survey

A start has been made on the suggested survey of the eyes of a variety of species of animals for use in irritant evaluation. As mentioned in the previous quarterly report, this work has been deliberately deferred in order to allow the project personnel to achieve a high degree of familiarity with the currently used procedures. The survey is now being carried on at a rate that will not unduly interfere with the on going screening operations. The tentative findings, to date are these. After a brief trial, the mouse has been rejected as unsuitable due to his small size. Although some difficulties are encountered in proper restraint of these animals, the principal difficulty is that the mouse eye is so small that there is extreme difficulty in detecting the details of the irritant effect. Similarly a limited number of trials with dogs permits some tentative conclusions. However, these are subject to possible revision when larger numbers of animals and a greater variety of compounds have been tested. Essentially, the same spectrum of signs are produced in the dog as in the rabbit. However, all signs appear to be less intense and to last for a shorter period of time in the dog than in the rabbit. The tentative conclusions are that: (1) the dog is somewhat less sensitive to eye irritation; (2) that the usual dark-colored iris in the dog makes examination somewhat more difficult, and (3) that the combination of blepharospasm and extension of the nictitating membrane which occurs in some dogs in the presence of irritation makes it difficult or occasionally even impossible to observe the iris and cornea.

B. Plans for subsequent period:

It has been decided to explore other tissues as sources of histamine in the hope that they may prove more sensitive to histamine liberation by irritants than appears to be the case with rat peritoneal mast cells. The next tissue to be explored is the guinea pig lung. The species survey will, in the very near future, be extended to include cats and certain species of monkeys. With respect to species of monkeys, effort will be made to obtain species with relatively light-colored irises for reasons previously mentioned. At a recent conference with the Contract Project Officer it was decided to initiate exploratory studies of ciliary activity and bronchial muscle (tracheal chain). Steps have been taken to acquire the necessary animals and equipment to follow the procedures described in the proposal.

PART II - Assessment of Irritant Potential

A. Compounds received:

Received 7/24/63

CS 9784
CS 23412
CS 24865
CS 24801
CS 28938
CS 31676
CS 31677
CS 31688
CS 39228
CS 39229
CS 39230
CS 39232
CS 39233
CS 39239
CS 39241
CS 39242
CS 40834
CS 40840
CS 40841
CS 40843
CS 40844
CS 40849
CS 40850
CS 40877
CS 41455
CS 41458
CS 41460
CS 41462
CS 41467
CS 41468

CS 41469
CS 41470
CS 41543
CS 41576
CS 41577
CS 41591
CS 41592
CS 41593
CS 41623
CS 42213
CS 42214
CS 42216
CS 42218
CS 42737
CS 42739
CS 42740
CS 42811
CS 42812
CS 42818
CS 42819
CS 42820
CS 42821
CS 42822
CS 42824

Received 8/22/63

CS 27644
CS 39651
CS 39654
CS 39666
CS 41722
CS 41723
CS 41724
CS 41725
CS 41726
CS 41727
CS 41763
CS 41864
CS 42039
CS 42049
CS 42054
CS 42055
CS 42056
CS 42057
CS 42064
CS 42066
CS 42069
CS 42224
CS 42354
CS 42355
CS 42356
CS 42982
CS 42983
CS 42984
CS 42985
CS 42986
CS 42987
CS 42990
CS 42997
CS 42998
CS 43000
CS 43001
CS 43054
CS 43157
CS 43166
CS 43168
CS 43169
CS 43175
CS 43176
CS 43178
CS 43179
CS 43180

B. Work completed:

Assessment has now been completed on the following compounds which were incomplete at the time of the first report.

CS 15414
CS 15442
CS 18639
CS 20409
CS 23654

Additional compounds on which complete assessment has been accomplished:

CS 9784
EA 118-055-5
EA 119-400-1
CS 19843
CS 23412
CS 24865
CS 25299
CS 28401
CS 31627
CS 31638
CS 31641
CS 31642
CS 31677
CS 34927
CS 34928
CS 36828
CS 36870
CS 36871
CS 37267
CS 37268
CS 37269
CS 37270
CS 38355
CS 38356
CS 38730
CS 38731
CS 38739
CS 39228
CS 39233
CS 39239
CS 39241
CS 39886
CS 39902
CS 39903
CS 40320
CS 40323
CS 40324
CS 40325
CS 40331
CS 40332
CS 40669
CS 40677
CS 40679

CS 40683
CS 40686
CS 40781
CS 40785
CS 40800
CS 40801
CS 40804
CS 40805
CS 40806
CS 40840
CS 40844
CS 41455
CS 41467
CS 41468
CS 41469
CS 41470
CS 41577
CS 41591
CS 41593
CS 41623
CS 41722
CS 41725
CS 41726
CS 41763
CS 41864
CS 42049
CS 42055
CS 42056
CS 42057
CS 42213
CS 42216
CS 42739
CS 42740
CS 42818
CS 42819
CS 42820
CS 42822
CS 42985
CS 42986
CS 43001
CS 43166
CS 43168
CS 43176

Attention is called to an error which has been discovered in the first report. Complete assessment was reported for CS 17593. This should read CS 17953

Compounds on which assessment is incomplete (evaluated for <u>skin</u> irritation only):

CS 31626
CS 39654
CS 40678
CS 40685
CS 40797
CS 40843
CS 41723
CS 42039
CS 42355
CS 42984
CS 42987
CS 42990
CS 42997
CS 43054

Compounds on which assessment is incomplete (evaluated for <u>eye</u> irritation only):

CS 28938
CS 39242
CS 40834
CS 40841
CS 40849
CS 40850
CS 41460
CS 41462
CS 41543
CS 41576
CS 41592
CS 42214
CS 42218
CS 42747
CS 42811
CS 42821
CS 42824
CS 42998
CS 43000
CS 43169

As provided in the contract, technical reports on this phase of the work have been submitted monthly to the Contract Project Officer.

C. Discussion:

By devoting the full time effort of a trained team to this phase of the work the equivalent of complete assessment of approximately 100 compounds has been accomplished (83 complete; 16 eye only; 24 skin only). This fulfills the prediction made in the first report. In fact schedules are now in operation which accomplish complete evaluation of approximately 15 compounds per week.

D. Work planned

On the basis of schedules which have been in operation during the last portions of this report period;we confidently predict complete assessment of the planned 200 compounds by approximately the middle of the third quarter of the contract.

SUMMARY

During the second quarter of the contract emphasis has been placed on assessment of compounds by current methods. Histamine liberation from rat mast cells has been found to be small and other tissues are being considered. New directions for methods development are being explored.

Assessment by current methods has been completed on approximately 100 compounds (83 eye and skin; 16 eye only; 24 skin only). Schedules now in operation will permit completion of assessment of the remaining compounds by the middle of the third quarter.