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FINAL TECHNICAL REPORT

under contract DA-91-591-EUC-2036

Performed during the period: November 1961-November 1962  
by the contractor: Karolinska Institutet  
Bakteriologiska Institutionen,  
Stockholm through Prof. B. Malmgren  
and in his absence: Ass.prof. T. Holme



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## I. SUMMARY OF SCIENTIFIC ACCOMPLISHMENT UNDER CONTRACT

The machine designed, calculated and manufactured under contract DA-91-591-EUC-1619; OI-7206-61 has been tested in co-operation with Dr. W. F. Daniels from Fort Detrick.

The physical and mechanical tests revealed that a heavy (112 kg without rotor and liquid) stainless steel rotor, cylindrical in shape, can be spun horizontally as a routine operation at speeds up to 15,000 r.p.m. on water lubricated bearings. The auxiliary devices permitting rapid starting and stopping and preventing heating of the test suspensions functioned very well. The bacterial cells (E. coli B) collected evenly in the cylindrical chambers at the periphery of the rotor, but, unlike in the light miniature rotor tested several years ago, larger amounts of cells tended to immobilize the suspending rods of aluminium. This was done so efficiently that only very violent motions would release them, thus permitting resuspension to take place. Since the situation was not improved by the use of heavier rods (stainless steel), a basic redesign of the rotor seemed necessary. Against this background the improvement of the sedimentation efficiency of the present rotor was only carried so far as was regarded useful to illustrate the main phenomena controlling the performance. Actually the efficiency was not pushed beyond 40 liters of E. coli culture per hour with a 95 % sedimentation efficiency. It was strongly influenced by three phenomena: pumping capacity, turbulence and foaming, each involving a number of parameters.

The experience gained in operating a horizontal rotor has been of great value, and a simplified rotor is now being designed to fit the housing and drive assembly used. It is shaped as a simple bowl, has a much larger sludge space and a single suspending roller retracted towards the center during sedimentation.

## II. REPORT ON RESEARCH PERFORMED

- Contents:
- A. Objectives of the contract.
  - B. Summary of the research performed.
  - C. Implications of results for future work.
  - D. Summary on personnel utilized and administrative actions taken.
  - E. Estimate of number of working-hours expended and of costs for materials used and for property acquired.

### A. Objectives of the contract.

The general objectives of the contract were to supply the information necessary to construct a centrifuge having a nominal maximum capacity of 8 liters per minute, while achieving a 25-fold concentration of cell suspensions. The apparatus should be safe and strong (safety design factor of 1,25 to 1,50 or better at 15.000 x g) and it should be tested on suspensions of bacteria and bacteriophages.

As will be shown in the following sections the performance of the apparatus did not come up to expectations, and the tedious search for optimum conditions had to be discontinued for many months due to a mechanical failure. However, the experiments have clearly shown that horizontal operation is a feasible design approach even in the case of heavy rotors and high speeds.

All relevant design information, including a set of drawings with English text has been provided to Dr. W. Daniels, who carried out or participated in most of the test runs. The manufacturing materials used were listed in the final technical report of contract DA-91-591-EUC-1619; OI-7298-61.

### B. Summary of research performed.

#### 1. Principle of apparatus (the numbers refer to the cross-section figure).

To serve as a basis for the following section, the general functioning of the machine must be summarized.

A suspension of microorganisms is introduced into a horizontal rotor (1) which is completely enclosed in a housing (24) which serves as a mechanical shield, an autoclave and a cooling jacket. The suspension enters one shaft of the rotor (17), as a liquid jet and is then distributed to 12 cylindrical chambers which lie at the periphery of the rotor body and parallel to its axis. Under the action of the centrifugal force the particles settle out in the chambers, but the supernatant flows out over a threshold (3) and escapes from the rotor via centrifugal valves (10,11) which open when the rotor spins at a speed which is above a certain critical value. The supernatant leaving the rotor is collected and removed from the machine.

When the particles are to<sup>be</sup> harvested the suspension feed is stopped, and a flow of buffer solution or other suitable medium for the resuspension of the particles is started. The speed of rotation is then reduced so that 12 solid rods (2), located in the peripheral chambers, start to roll, thus effecting the suspension of the sediment. The suspension, which cannot escape through the centrifugal valves at low speed is finally removed from the machine by means of a syringe, the needle of which is pushed through a rubber membrane (14) covering an opening in the shaft. The opening leads to a channel (6) which goes down into the suspension.

## 2. Functional tests.

### a. Introduction.

As indicated in last year's report the choice of bearing material was important. Bearings both of Kufalit and Ferrobestos were manufactured, and the latter, which were found to be satisfactory were used throughout the tests.

It was found that the Ferrobestos bearings swelled somewhat when boiled (1 o/oo<sup>diam</sup> change after 2 + 3 hrs' boiling) a fact which did not however prevent their being used as steamsterilized bearing sleeves in the pilot plant fermentors. The Kufalit proved to be too brittle. A special test shaft was manufactured to try out some novel bearing materials, which were expected to become available towards the end of 1962.

The only bearing failure occurred in June, and was caused by the waterhose carrying lubrication water to the front bearing, coming loose unnoticed. This happened at full speed, so the bearing burnt out in a matter of seconds. Before the rotor stopped, its main body had suffered a cut from the effluent collector ring (29), one of the shafts (21) had been bent and its bearing surface badly damaged. The repairwork plus the rebalancing made necessary by this misfortune took several months and of course upset the testing program. The time necessary for the machinework on the rotor could however be utilized for providing the apparatus foundation with a vibration damping which previous runs had shown to be desirable. Also the time could be used for general servicing, painting etc. and for adding some fixtures like belthousing, permanent piping, working platforms etc.

### b. Physical testing.

First the rotor was balanced by the removal of 10 g of material. Thus the unbalance was reduced first to 12,5 gcm and in a later operation to a final difference between the center of gravity and true center of axis of about 1  $\mu$ . This was well below the permissible maximum and so was also the weight difference of the rods (less than 0,1 g). The machine was fitted with Ferrobestos bearings and those were provided with lubrication water from two 1,2 mm diam. orifices in a 6 kp/cm<sup>2</sup> water line (1 l/mi. each). The tests began with aluminium rods and slow speeds with incremental speed build up and examination prior to runs at higher speeds. Also the bearing temperature was monitored with thermocouples embedded in the material and the effluent water temperature was continually checked. The machine was run to a final speed of 14.200 r.p.m. with the aluminium rods and to 12.200 r.p.m. with the stainless steel rods without the development of any undue structural flows, the stresses being estimated with a MIT "stress-coat". A number of tests were performed, and they gave the following main results. (A detailed 13-page protocol with temperature graphs etc. was prepared). The numbers refer to the cross-section figure.

- a. A selfadjusting thinbelt drive of the type employed (SESPA-ASEA; SM 25 L-MARC 15 with dacronreinforced Solufus-belts, type 1251 from Antriebe AG, Kapperswil SG, Switzerland) was well suited for operating at 15.000 r.p.m. and for accelerating the heavy rotor body (empty 112 kg) to full speed in about three minutes with the aid of a 12 Hp 2900 r.p.m. motor (380 V; 50 Hz) controlled by a starter with 15 - 25 A thermic relais (ASEA DEF 25 + PBA 1016). Two alternative pulleys were employed. The current used during the acceleration was followed on a wattmeter.
- b. The rotor housing combined a good mechanical protection with excellent cooling and overhauling of rotor and bearings could easily be effected thanks to the fact that the whole machine could be tilted to an upright position.
- c. The brake (in the form of the disc 19 which could be immersed in water) proved to be an excellent deceleration device particularly in the range above 2000 r.p.m. It cut the time for stopping the rotor to less than five minutes.
- d. The principle to let the horizontal shafts float on a water film in the bearings was sound, and the wear during acceleration and deceleration, i.e. before and after the liquid film was formed, proved to be insignificant. The minimal filmthickness was calculated to be 27  $\mu$  at

12.000 r.p.m. and a diametral difference between shaft and bearing of 0,12 mm. It would decrease if the diameter difference increased or the speed was reduced. The monitoring of the power requirements and fluctuations revealed a variable load below 1200 r.p.m. which was thought to denote a water film break-up within the bearings. This speedrange should thus be avoided for longer than necessary to build up to higher speeds or to drop to lower speeds.

- e. A design based on cylindrical chambers parallel to the axis of a horizontal rotor (1) was mechanically feasible and permitted an evenly distributed liquid flow through the machine. Since different granular materials can be distributed evenly in the chambers by weighing and can be retained by a high threshold (3) this fact offers interesting possibilities for experiments on centrifugation combined with filtration or passage through an ion exchange bed (viruses !).
- f. Charging the cylindrical chambers with loose aluminium or stainless steel rods (2) only slightly affected the vibration pattern and in no way interfered with the acceleration, running and deceleration of the rotor. The aluminium bars at first quickly corroded but after anodizing they showed no signs of surface deterioration.
- g. The vibration pattern followed by means of a pickup (Philips PR 9250) connected to an oscillograph (Philips G4 3156) showed no conspicuous differences when the machine was run empty or filled to capacity with liquid.
- h. The ice-water cooling (+ evaporation of bearing water ?) removed so much heat that a liquid passing through the machine only increased in temperature by a few degrees centigrade. The fear expressed in the final technical report covering the work during 1961, where running in a helium atmosphere was considered as a possible necessity, was found to be unwarranted. Passage of the rear bearing lubrication <sup>thus</sup> water between the stator and the rotor was shown to <sup>have</sup> a considerable cooling effect by itself. In a case where there was no cooling of the interior stator wall the rotor temperature was found to be only 40° C after an hour and a half of running.
- i. The unloading valve and springs (9,10,11) were found to function satisfactorily when the opening pressures had been reduced to 5 kg/cm<sup>2</sup>.
- j. The effluent collector ring (29) did not prevent spill-over into the bearing water drains and should be redesigned in later models. Also the rotor endplate should be redesigned since stresses were detected around the

screwholes and around the centrifugal valves. A further improvement would involve thicker lips on the bearing sleeves in order to reduce the axial free mobility of the rotor (2 mm).

### c. Microbiological testing.

The main test material was E. coli B, but also phage T2 was used in a few runs. The large scale cultivation of bacterial cells was routine, but for the production of phage the aeration had to be increased above the levels previously employed. With this in mind a new sparger was designed. It consisted of three concentric rings of stainless steel piping located in a horizontal plane below the propeller of the bottom stirrer. Each ring carried eight double Pyrex glass spargers (20 mm long, 10 mm in diam., 40  $\mu$  pore diameter). The rings were joined by radial connections and the most peripheral one (diam. 42 mm) was joined by four vertical pipes to a similar ring about 60 mm above it. Baffle plates were attached to the vertical pipes and an air hose to the upper ring. Sulphite oxidation tests gave an OR-value of 240 mm O<sub>2</sub> per liter and hour in 500 l of liquid and phage runs gave excellent yields. In connection with the latter the common laboratory Sharples was tested and found to be incapable of separating out the phage. Later dye-test experiments made it likely that the unsatisfactory performance of the Sharples machine was caused by a strong tendency to laminar flow which would cause a considerable portion of the suspension to follow a central path. Since such a tendency might also influence the performance of the horizontal rotor centrifuge, experiments aimed at changing the flowpattern mentioned were carried out on the Sharples machine. A row of cylindrical aluminium cups (4 by 4 cm) with the opening facing upwards were stashed into the rotor which they fitted exactly. Each cup had small holes drilled in its bottom, one in the center (acting as an air vent), and a circular row as close to the wall as possible. The rotor balanced perfectly at full speed even in cases where each cup was filled with 66 g of minute glass beads (ballotinis) used to reduce the sedimentation path of the phage particles. Only a few runs were performed, but they clearly showed that a much improved particle sedimentation could be attained, the only disadvantage being that only small amounts of material could be sedimented before clogging of the peripheral holes occurred.

The microbiological testing involved some forty runs and the main results are summarized below (the numbers correspond to the fig.):

- a. The vibrational pattern followed oscillographically was identical when the rotor was started, and when it contained a large amount of cells, indicating a homogenous distribution of sediment (confirming visual observations).
- b. The distribution of the sediment was not disturbed by the rapid deceleration effected by the water brake. The sediment extended from the threshold and down to 30 - 40 mm from the bottom of the chambers indicating a considerable turbulence at this level.
- c. Since the sedimentation efficiency as a rule was much better during the first part of a run than later, when a sediment had collected, it was thought that a "turbulence layer" became important when the sediment had reached a certain thickness. The presence or absence of the rods of course affected the holding time, but those did not seem to influence the suspected turbulence. Running the machine without rods, but with a semicircular wall (front end of a brass cylinder cut through its axis) in front of the bottom turbulence zone did not improve the performance, and neither did moving the peripheral chamber wall at this location towards the center (with the aid of a rubber disc). However, additional experiments with a complete sealing of the bottom turbulence zone by means of a disc having a small drainhole at the level of the threshold (3) would be desirable.
- d. The fact that a holding time of only 6 sec. was measured by dye injections at a flow rate as low as 20 l/hr indicated the existence of the same tendency towards laminar flow as was observed in the Sharples machine (see above). An excellent separation efficiency during the first 5 - 10 minutes of operation in spite of this short holding time shows that the gravitational field in the machine is adequate but that the flow pattern is not. Several experiments aimed at changing it were performed, for instance running the machine with a threshold (3) which was so high that the chambers were entirely filled with liquid. The high threshold was also used in an experiment where about 450 g of 7 mm stainless steel balls per chamber were tried out for suspending the cells. The vibration pattern was however so disturbed that this test could not be completed.
- e. Quite unexpectedly the suspending of the cells with the aid of the rods (2) proved to be difficult because larger quantities of bacteriaglued both aluminium (575 g each) and stainless steel rods (1575 g each)

so firmly to the periphery of the chambers that only very violent motions, which were difficult to effect, released them. In this respect the little aluminium test rotor had offered no problems. It is possible that approaches like the one mentioned under d. might eventually be successful, but the sludge space would be small. With this limitation in mind, it was regarded as the most rational approach to redesign the present rotor as soon as the main factors controlling its efficiency had been outlined.

- f. It was found very early that the pumping capacity of the rotor was of great importance for the efficiency. If the connection between the space outside the bottom shaft (17) and the main rotor housing was closed a negative pressure of 1000 mm H<sub>2</sub>O or more could be measured in the feed nozzle (28) or in the gas inlet of the space (used as bearing water drain after turning the cover /25/180°). By reducing the opening in the centrifugal valve (11) to 0,3 mm the airpumping was reduced considerably and the sedimentation performance improved.
- g. A factor of the greatest importance for extending the initial period of efficiency during a run was found to be chemical control of foaming (DC Anti-foam).
- h. In addition to the factors mentioned the location and design of the opening of the feed nozzle (28) within the shaft, the height of the threshold (3), which affected the liquid retention time, and of course the feed rate were all parameters, in controlling the sedimentation at a given speed. An extremely complex situation obviously existed making true optimization beyond 95 % sedimentation of a dense coli culture at 40 l/hr a matter of years rather than months. The value of such an effort seemed dubious, because the results would concern a very special design with obvious limitations (cf. e in this section). However, a continuation of the studies on the present will continue in parallel to the development of a new rotor.

### C. IMPLICATIONS OF RESULTS FOR FUTURE WORK.

The drive and bearing arrangements used and the cooling and general design of the housing have proved their value for operating a heavy (120 kg) horizontal stainless steel rotor (cylinder with length 45 cm and diam. 26 cm, containing 12 aluminium rods) in the 10.000-15.000 r.p.m. range. The relatively small sludge space

and unsatisfactory performance of the suspending rods, however, made optimization beyond a capacity of 40 l/hr seem unpracticable and a new rotor was designed. However, the present unit, which may become useful for studies on gravitational sedimentation in porous materials or ion exchange beds might be improved in many ways, for instance by sealing the culture inlet from the space surrounding the bottom shaft. This can be done by welding a stainless steel bellows carrying a carbon ring to the plate in which the feed nozzle is threaded. Also the liquid path in the chambers can be threefolded by redesigning the threshold (3) to permit every second chamber to act as a reverse path for the culture permitting it to enter its final travel to the front via a tangential connection drilled between certain chambers at the bottom of the rotor. Of course also eight of the radial inlet channels would have to be plugged.

The new rotor will be greatly simplified because it will take the form of a hollow cylinder. This will probably be made of titanium (ICI Titan 314 A Zugfestigkeit 97,67 kg/mm<sup>2</sup>, 0,1 % Dehngrenze 89,77 kg/mm<sup>2</sup>, Ermüdungsgrenze 60 - 65 % der Zugfestigkeit) which has good corrosion resistance and permits operation at even higher speeds than those employed. Running the waterlubrication system at higher speeds (possibly with glycol added) will be of considerable interest. The suspending of the cells will be effected by a roller which will be lowered from a position near the axis when the speed is reduced. With this approach it should be possible to reduce turbulence and to obtain a very large sludge space.

It is presupposed that the work will continue in close contact with Dr. Daniels and that the whole project, when finished, will be published in collaboration with him and Professor Boestad.

#### D. SUMMARY OF PERSONNEL UTILIZED AND ADMINISTRATIVE

##### ACTION TAKEN

The work has been carried out on a parttime basis by Professor G.K.U. Boestad, Mr. U. Dahl and Mr. J. Roos of the Royal School of Engineering, Stockholm, where most of the physical tests were performed and by Mr. B. Törnquist and Mr. S. Karlsson who provided drawings and coordinated the manufacturing of parts. Mr. Sager of the workshop at the Bacteriology Department, Karolinska Institutet, where the microbiological tests were carried out, also participated in the work. Finally Dr. W.F. Daniels from Fort Detrick took part in the tests, essentially on a whole-time basis, during the period October 1961 - June 1962.

E. ESTIMATE OF NUMBER OF WORKING HOURS EXPENDED AND OF  
COST FOR MATERIALS USED AND FOR PROPERTY ACQUIRED.

Besides the number of working hours spent at no cost to the contract by the principal investigator and by the pilot plant personnel of the Bacteriology Department, the following help has been hired under this contract and under DA-91-591-EUC-1619; oI-7206-61:

Professor Boestad	Sw. Cr. 3.649:--	) qualified
Mr. Roos	" " 800:--	) personnel:
Mr. Törnquist	" " 6.306:--	) about 400 hrs.
Mr. B. Seger	" " 543:--	) workshophelp: about 35 hrs.

Sw. Cr. 11.298:--

Material paid so far  
under the contracts

" " 17.642:--

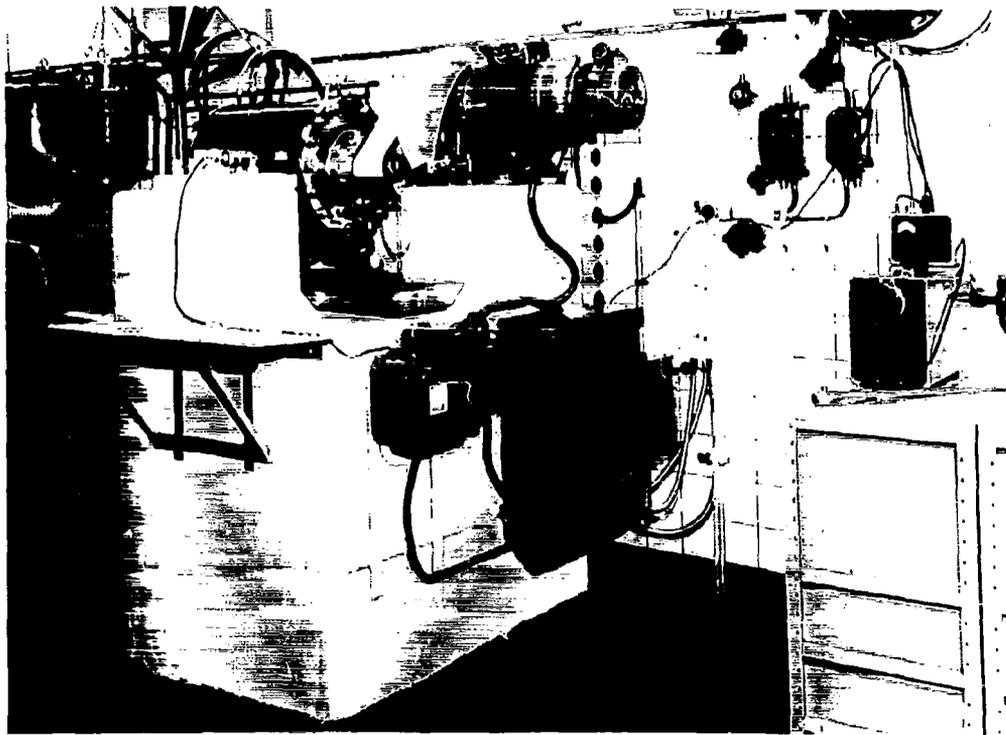
Expected invoices covering costs to date for machining of rotor with parts, heat exchangers and media used for microbiological tests

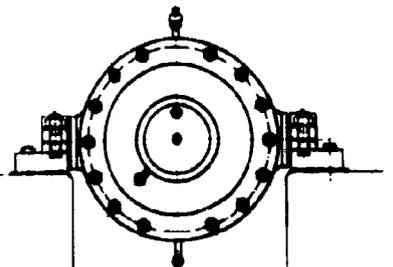
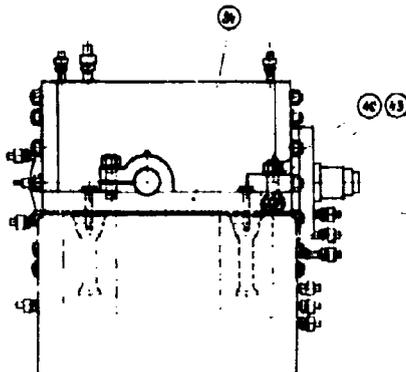
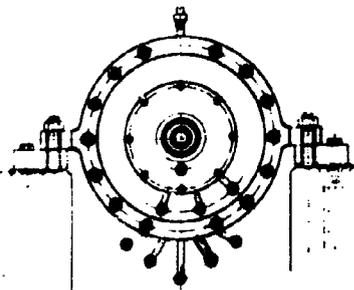
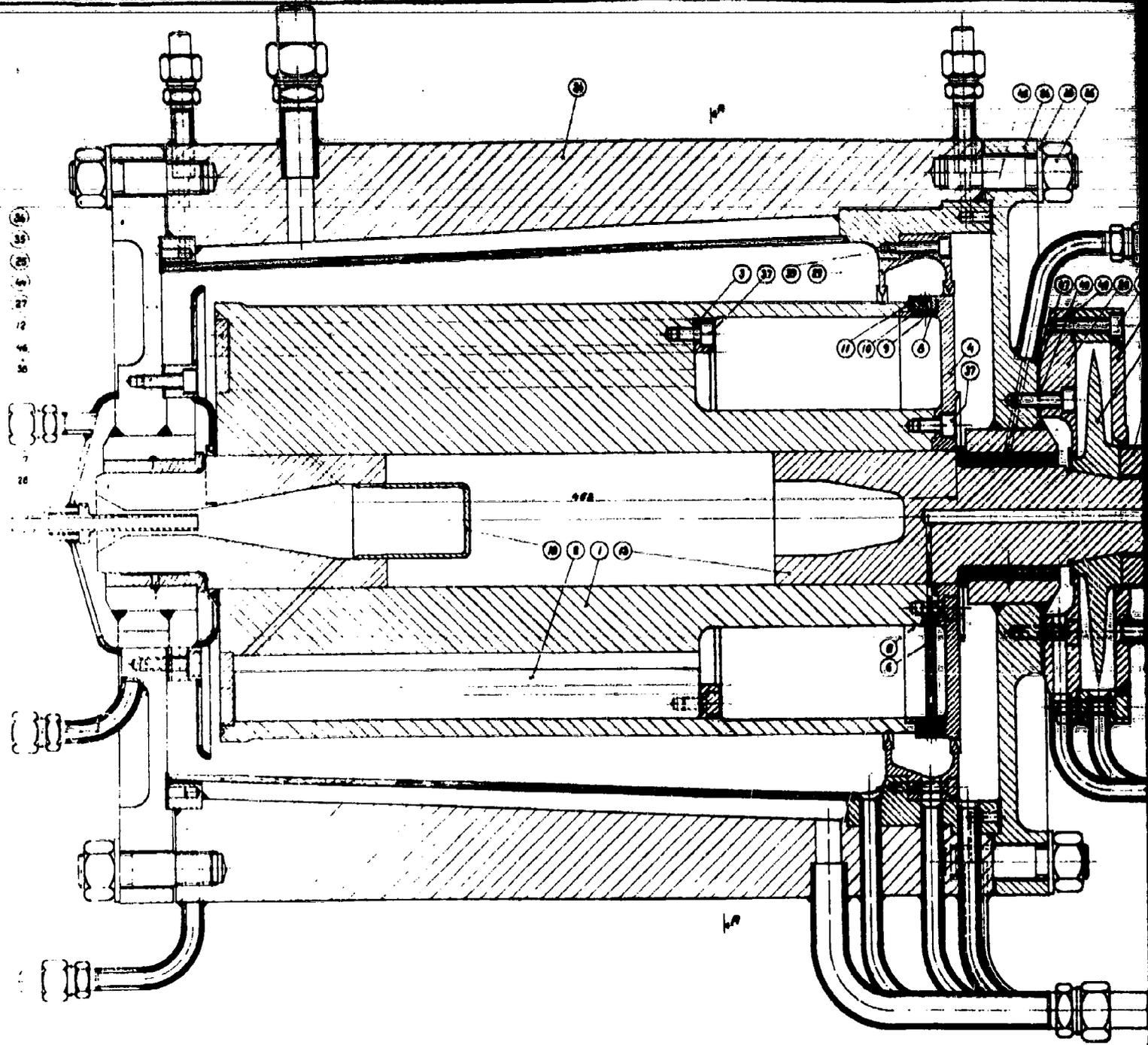
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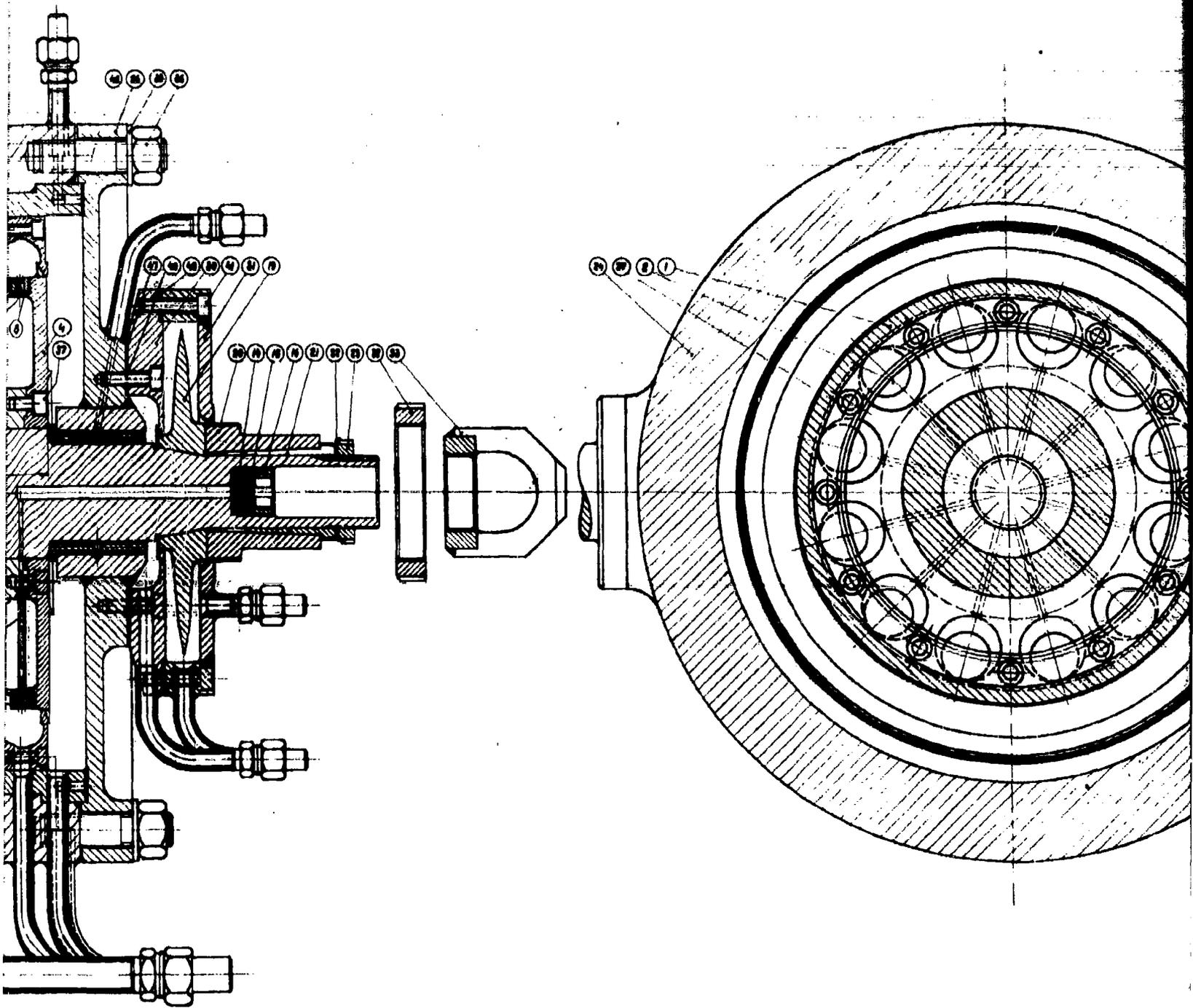
Stockholm, November 29th, 1962

  
C.-G. Hedén

Principal investigator  
under contract

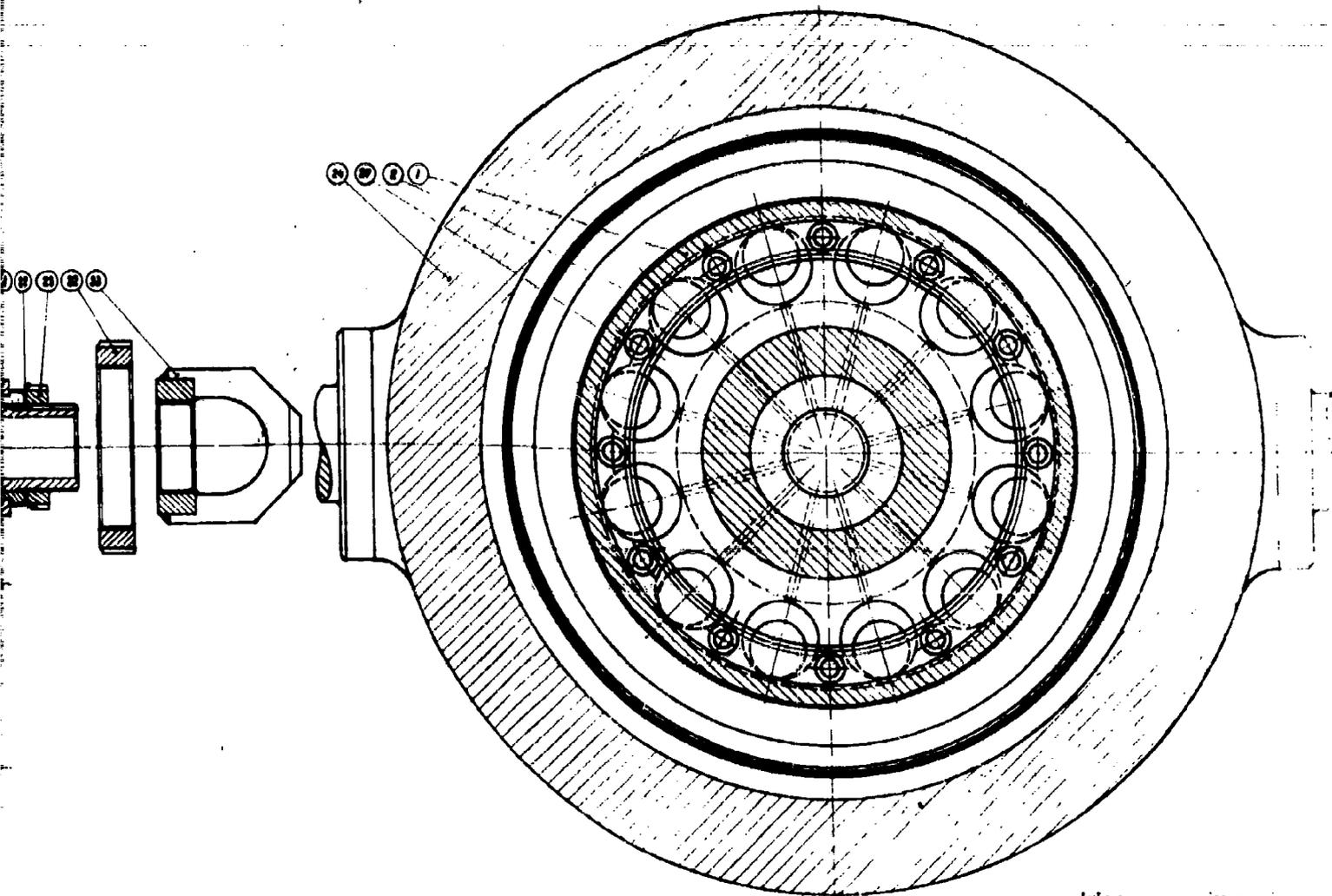






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