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Poo et al.

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(54) **ORBITAL SHAKER FOR CELL EXTRACTION**

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(22) Filed: **Feb. 24, 2003**

(65) **Prior Publication Data**

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Related U.S. Application Data

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(51) **Int. Cl.**
B01F 11/00 (2006.01)

(52) **U.S. Cl.** **366/187**; 366/216; 366/218

(58) **Field of Classification Search** 366/209, 366/210, 211, 212, 216, 217, 218, 208, 187, 366/188; 422/99, 184.1, 209, 224

See application file for complete search history.

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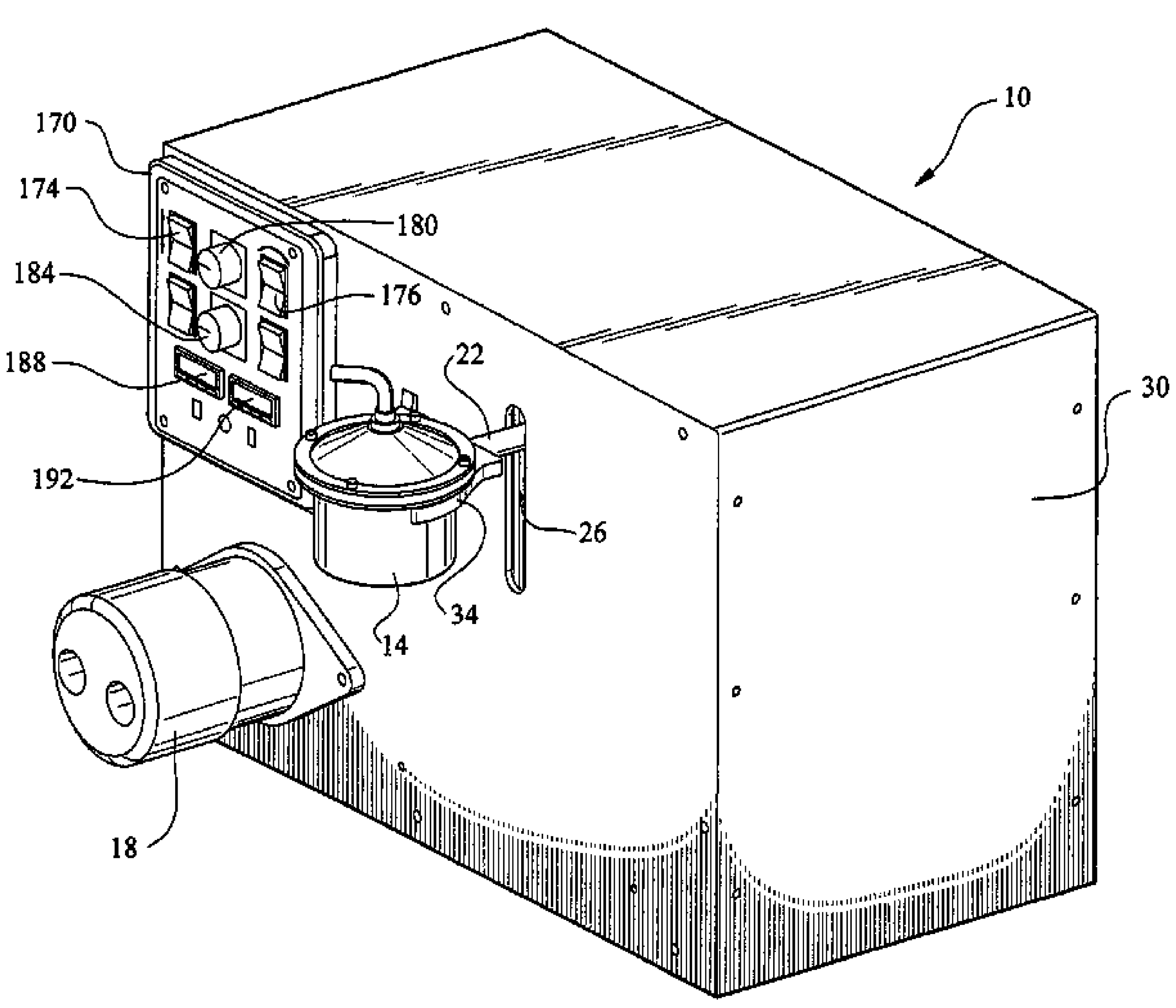
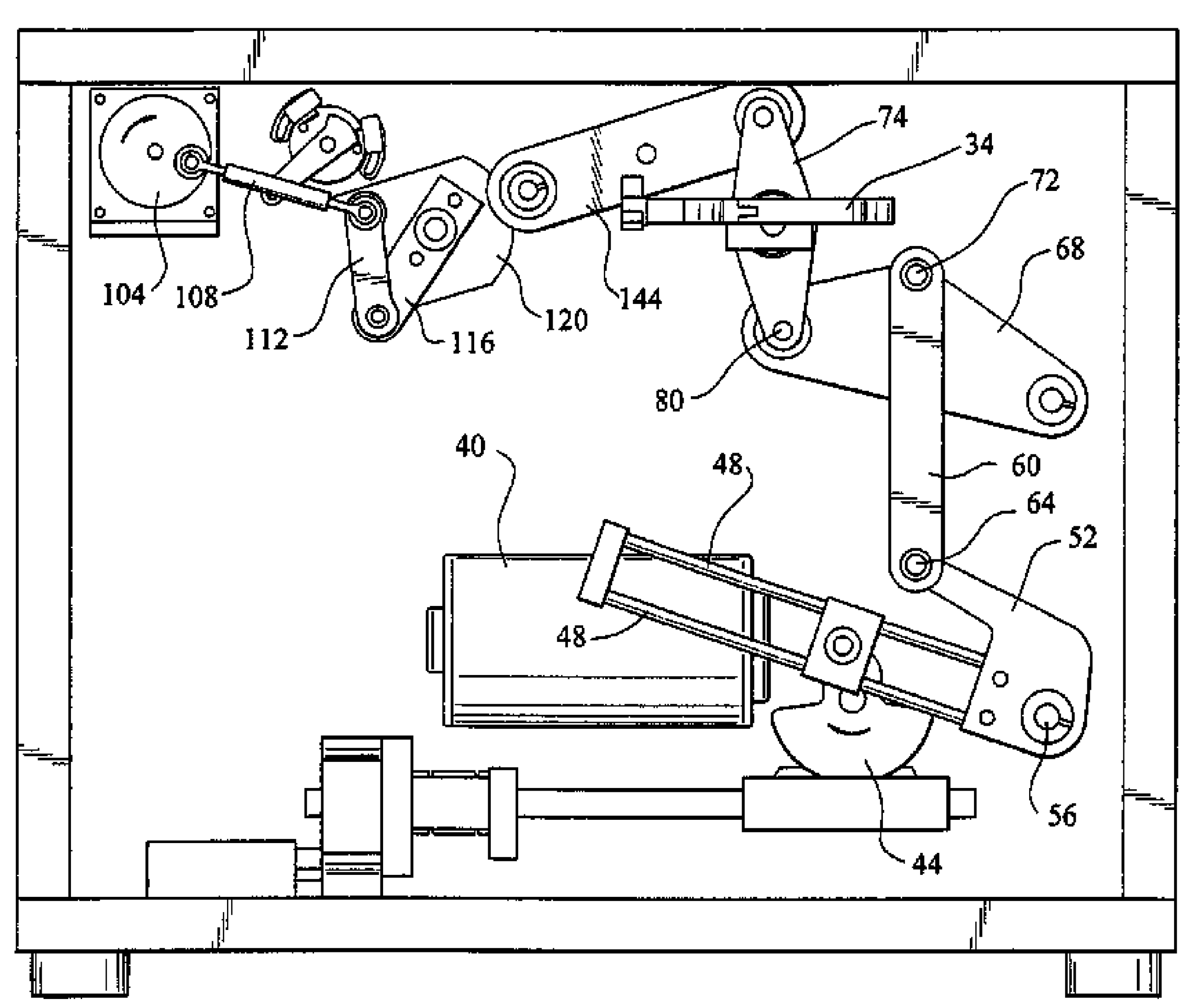
(57) **ABSTRACT**

A orbital shaker for cell extraction includes a digestion chamber and structure for translating the digestion chamber. Structure is also provided for rotating the digestion chamber. A method of performing cell extraction is also disclosed.

11 Claims, 8 Drawing Sheets





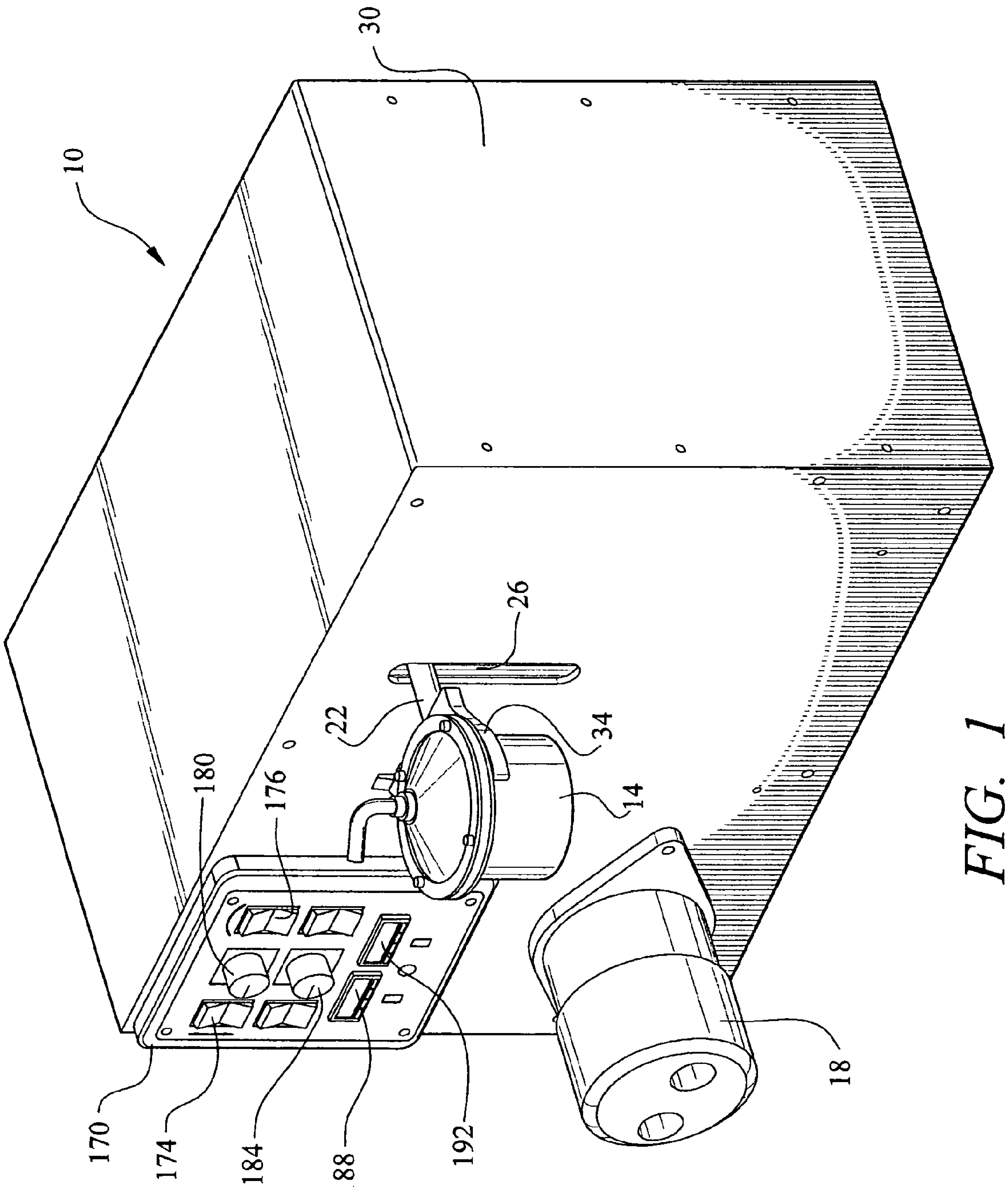


FIG. 1

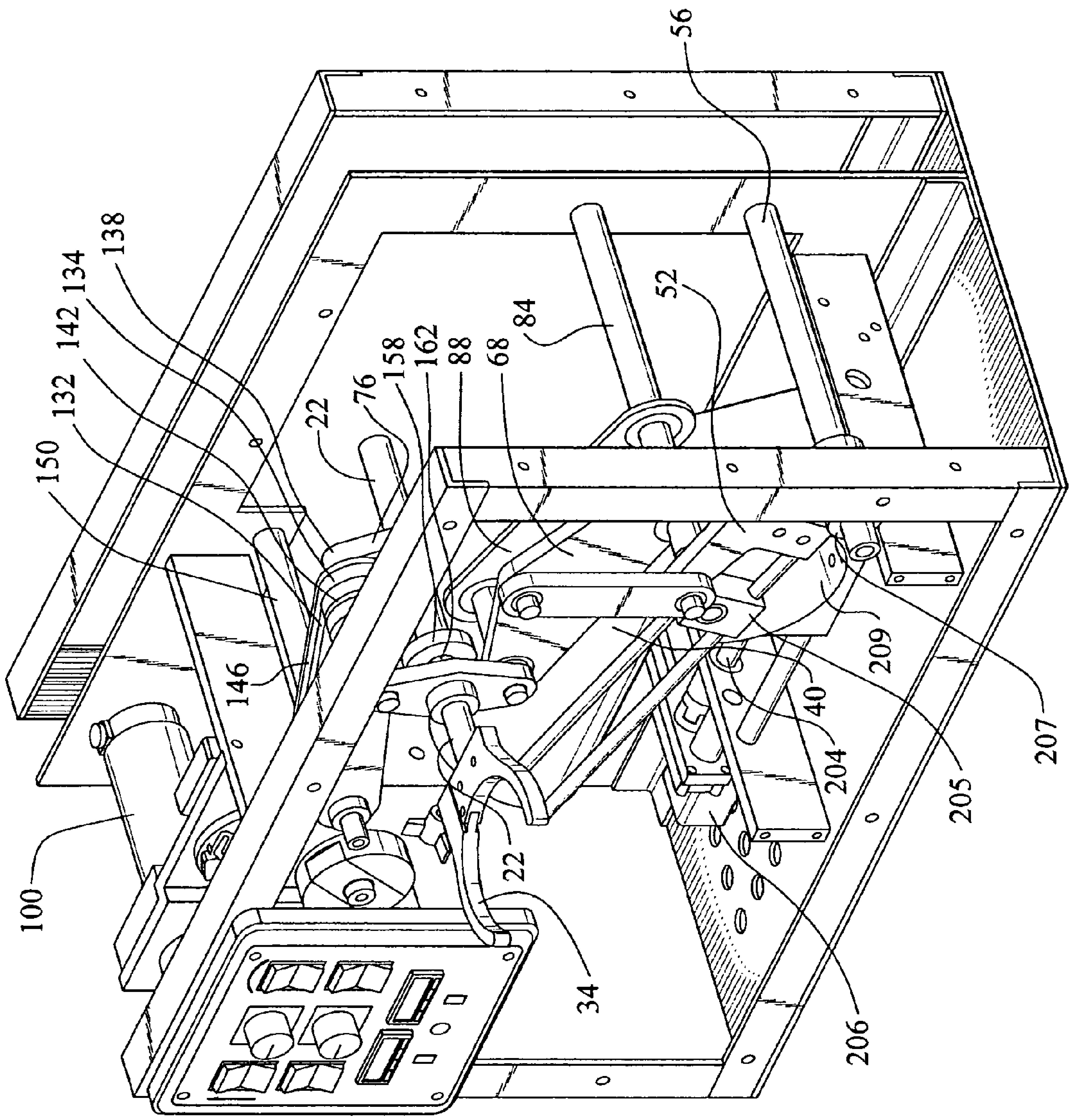


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FIG. 2

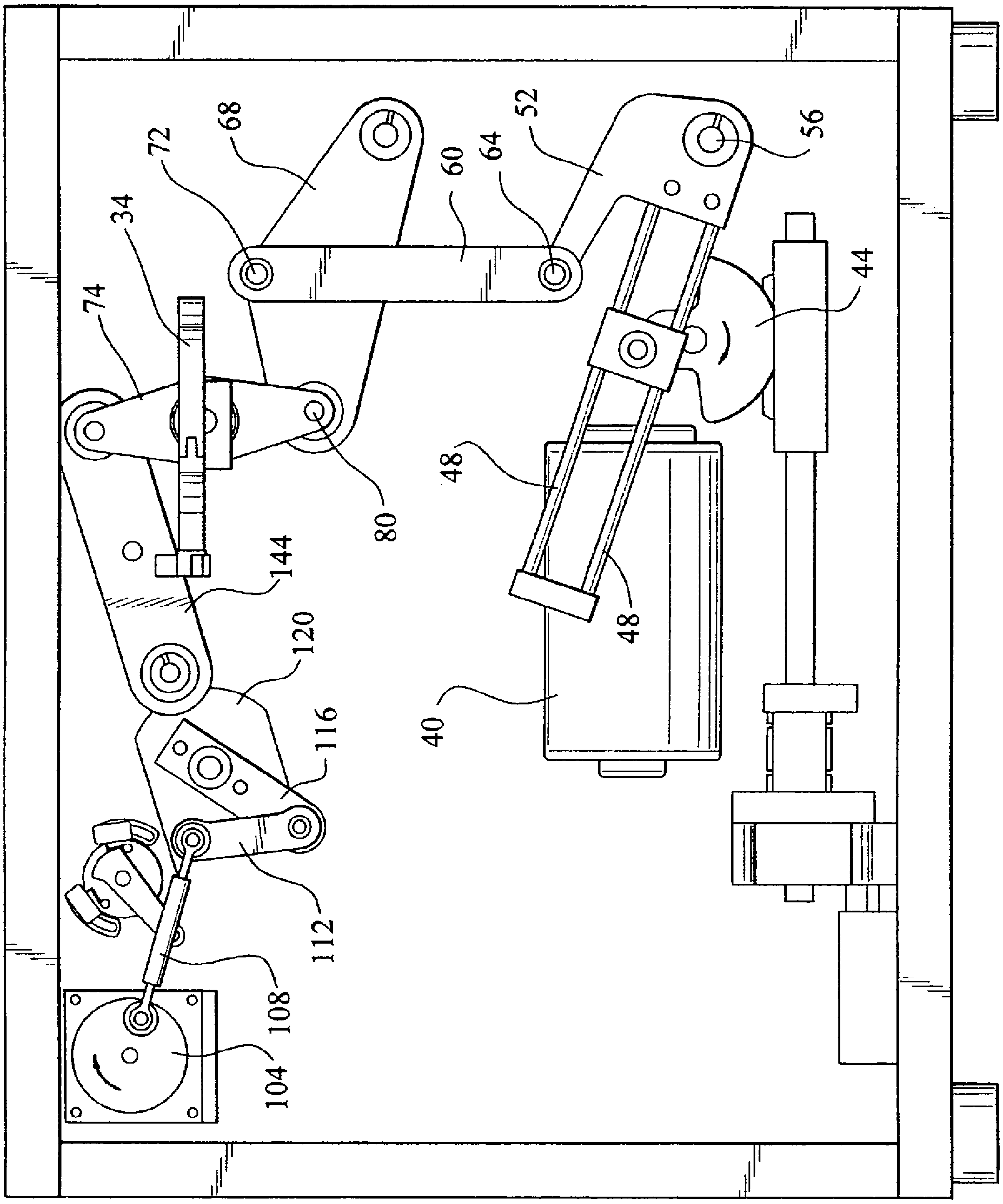

FIG. 3

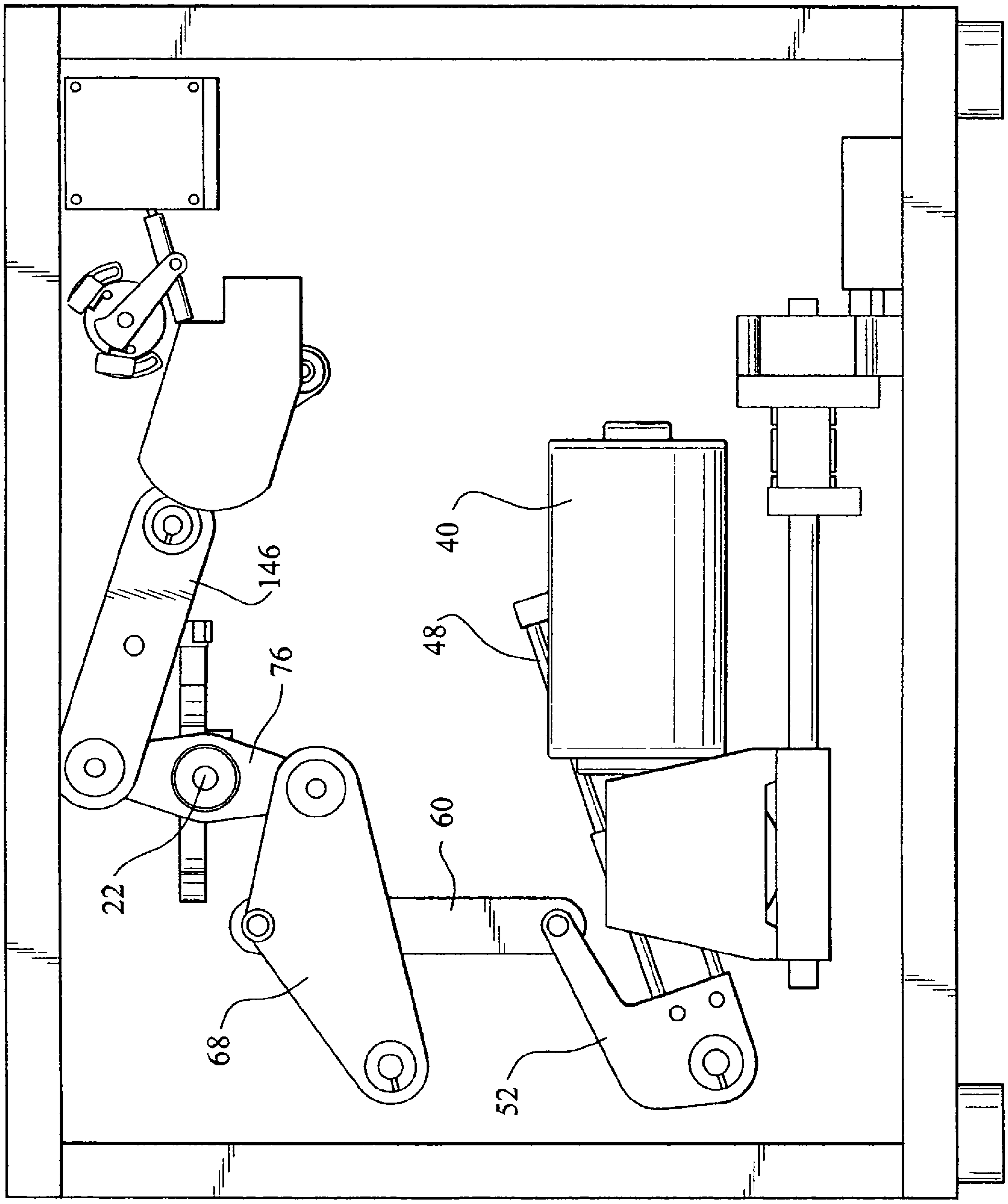

FIG. 4

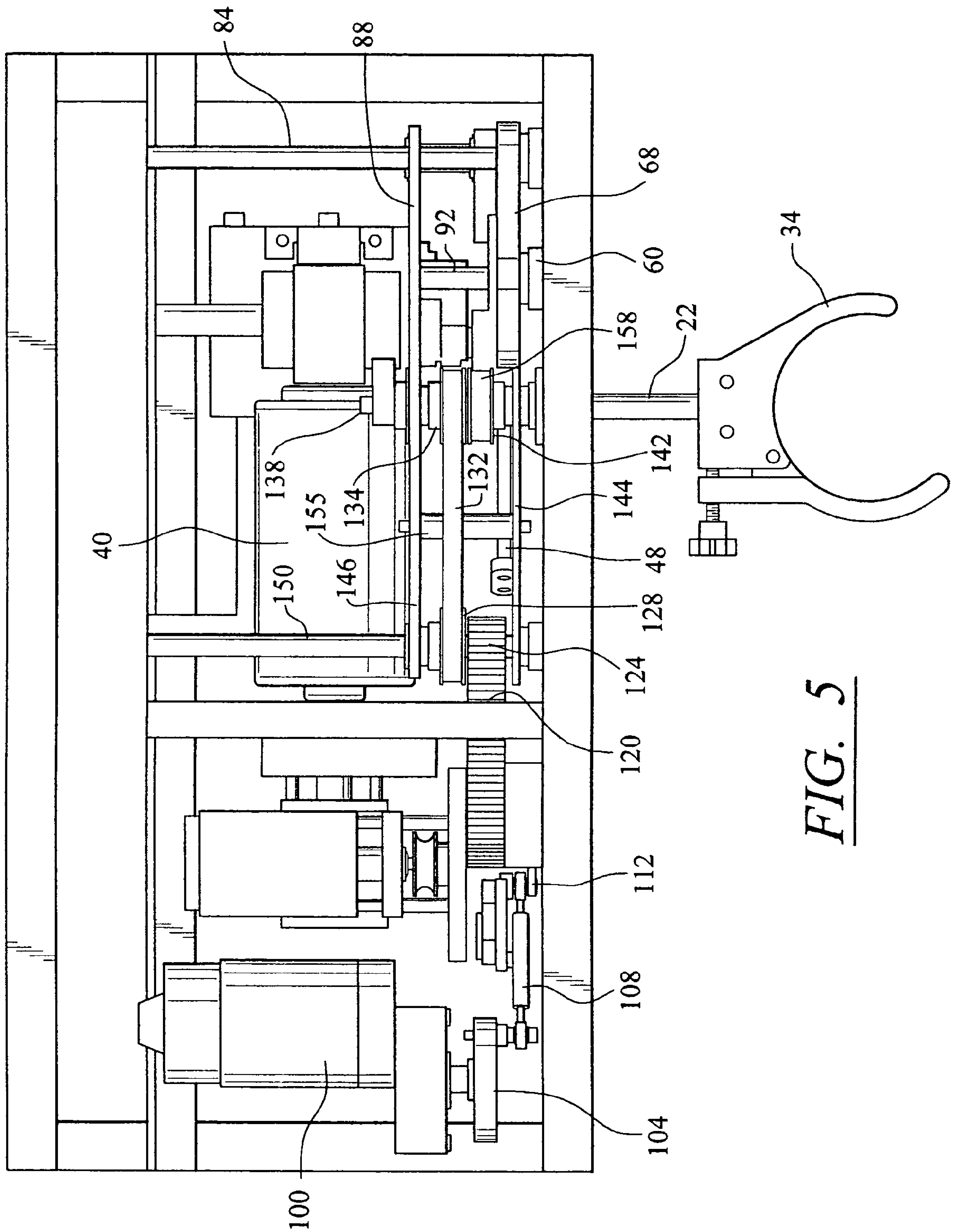
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FIG. 5

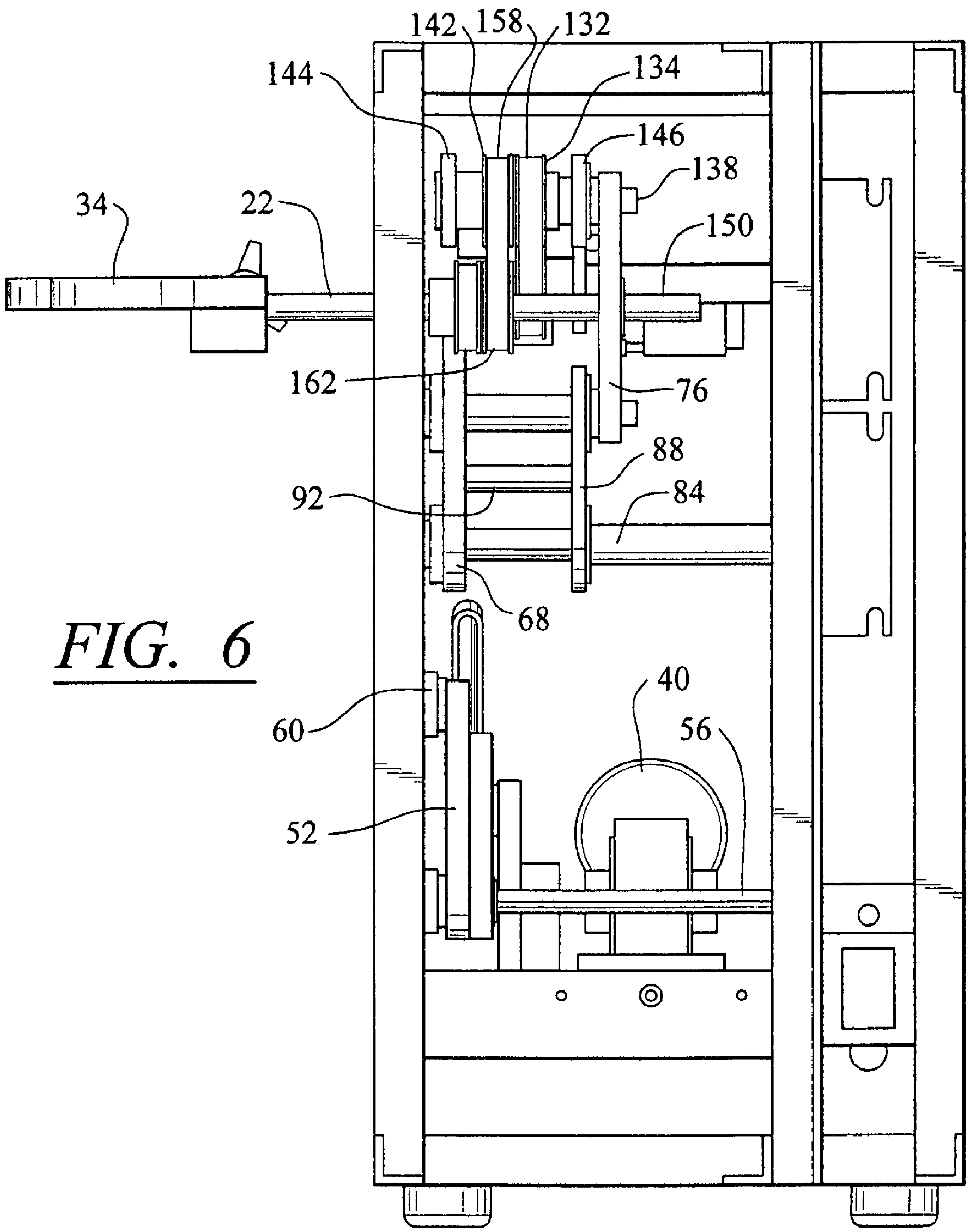

FIG. 6

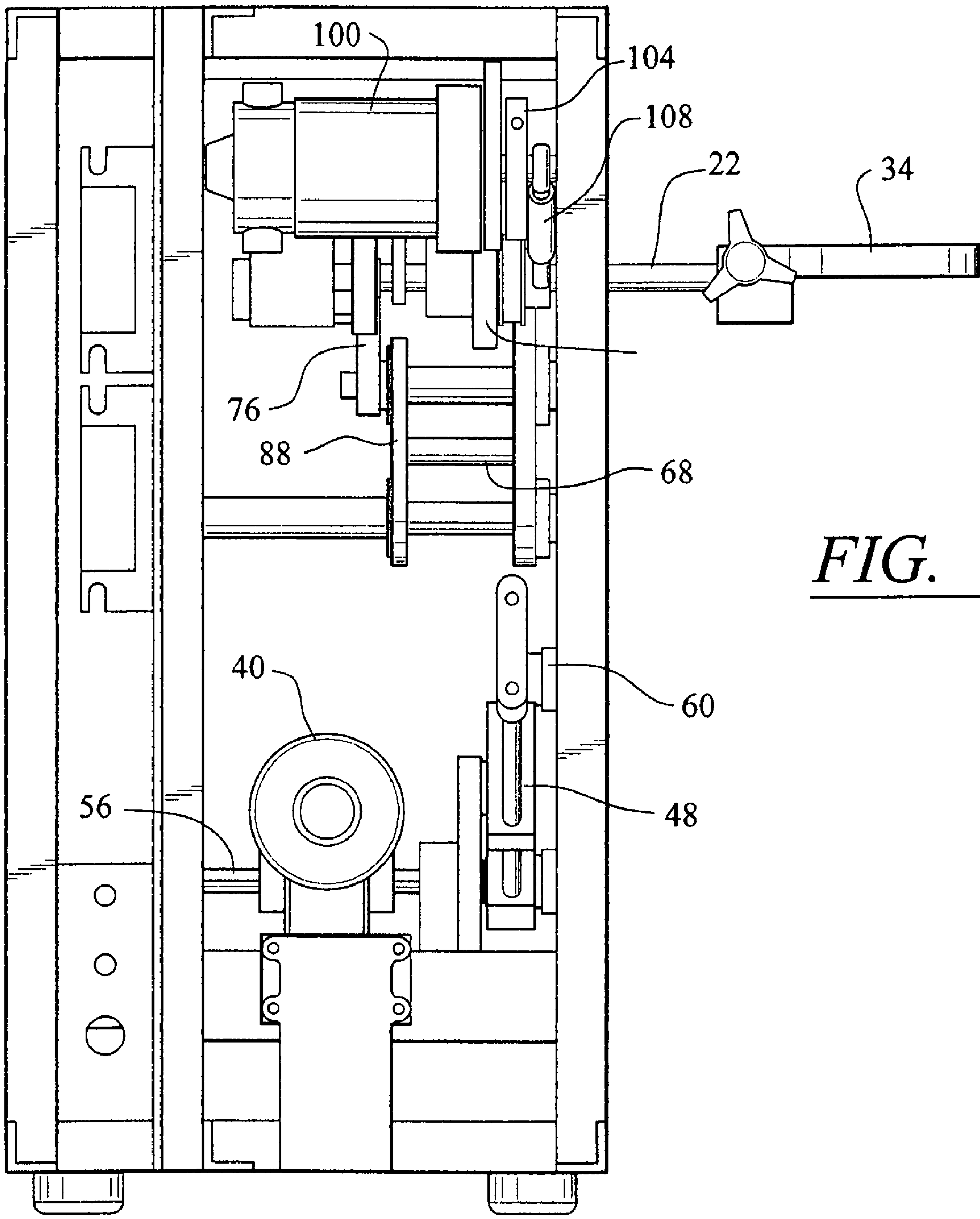

FIG. 7

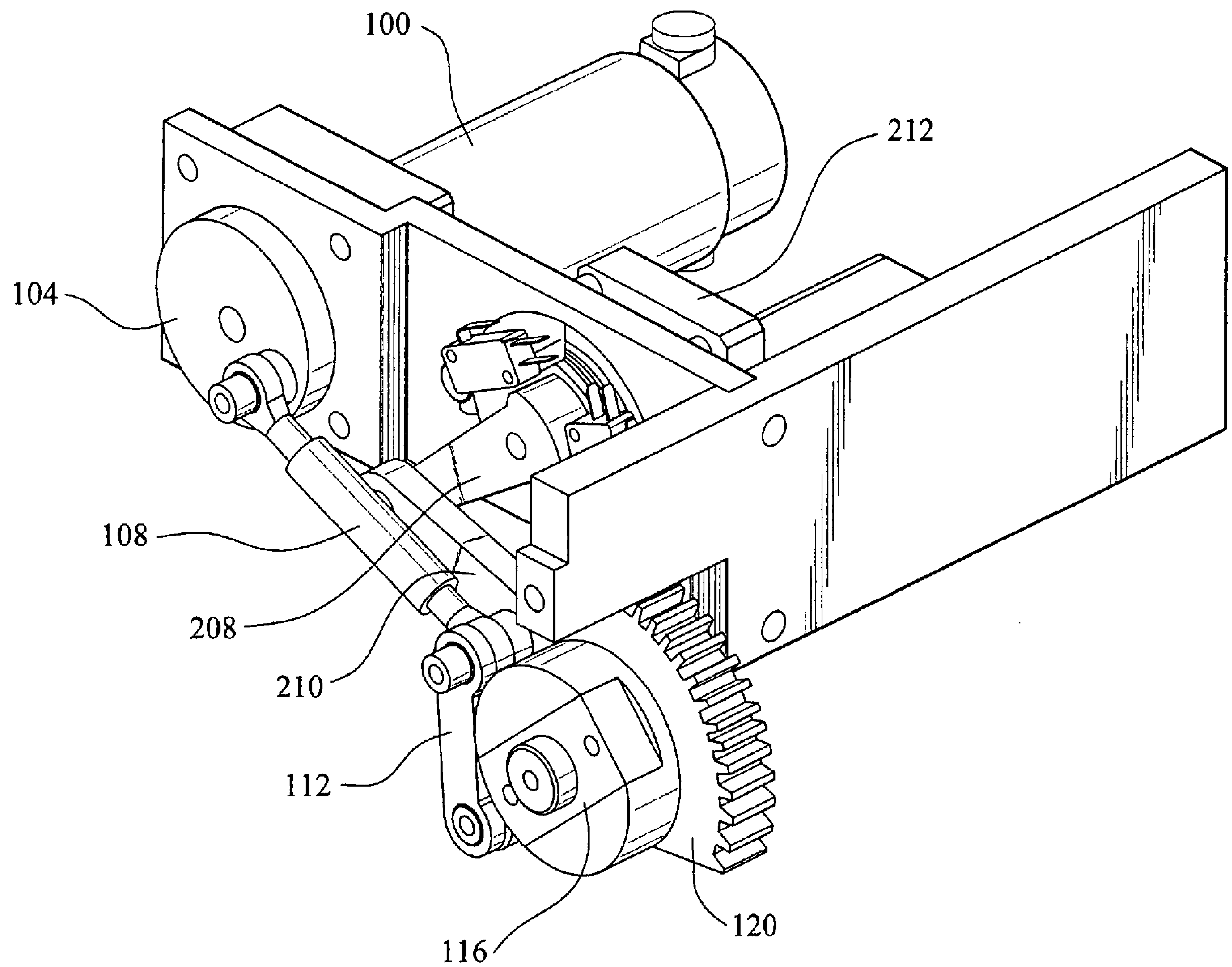

FIG. 8

ORBITAL SHAKER FOR CELL EXTRACTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Application No. 60/359,298, filed Feb. 22, 2002. The foregoing is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

BACKGROUND OF THE INVENTION

Scientists are currently researching possible applications for isolated cells from parent organs, such as the liver, spleen, kidney, adrenal, and pancreas. Some research that has been conducted on the clinical application of isolated cells has involved groups of cells called the Islets of Langerhans that have been isolated from the pancreas. An application for the Islet of Langerhans cells is as a treatment for diabetic patients. Patients with diabetes have Islets of Langerhans that do not function properly, and therefore, do not produce enough insulin. Some clinical research is aimed at developing a procedure for transplanting functioning Islets of Langerhans into diabetic patients to restore the insulin producing ability of the pancreas. Clinical research of such requires isolated Islet of Langerhans cells, but these cells must be isolated while still viable. Viable isolated cells are mostly obtained from organs of the very recently deceased. The apparatus and method for isolating the cells should be able to extract isolated cells with as little damage to the cells as possible.

Many different methods and approaches have been attempted to isolate individual cells from their respective parent organs. Prior methods have produced isolated cells with some cell destruction. This cell destruction can result from the relatively severe mechanical stimulation that is used to isolate cells from an organ.

One method that attempts to overcome the loss of damaged cells due to relatively severe mechanical stress is described in U.S. Pat. No. 5,079,160, to Lacy, et al. The method disclosed by Lacy, et al. comprises the steps of: placing an organ or a piece of an organ in a digestion chamber along with marble agitators; distending the organ or a piece of the organ with physiologically compatible medium containing a protease; continuously recirculating that medium; and separating the isolated cells. The marble agitators greatly increase the amount of undamaged cells obtained through isolation without reducing the quality of the isolated cells obtained by gently agitating the organ. Moreover, the marbles are an appropriate size, weight, and density for obtaining beneficial results as compared to other agitators of varying size, weight, and density which can cause severe mechanical disruption of the organ tissue resulting in some cells being destroyed.

SUMMARY OF THE INVENTION

An orbital shaker for cell extraction, includes an engagement structure for engaging a digestion chamber; a translational drive structure for translating the digestion chamber; and rotational drive structure for rotating the digestion chamber.

A method for cell extraction includes the steps of providing tissue in a digestion chamber; supplying a digestive medium to the digestion chamber; and using an orbital shaker apparatus to translate the digestion chamber while rotating the digestion chamber.

BRIEF DESCRIPTION OF THE DRAWINGS

There is shown in the drawings embodiments which are presently preferred, it being understood, however, that the invention can be embodied in other forms without departing from the spirit or essential attributes thereof.

FIG. **1** is a perspective view of a orbital shaker for cell extraction.

FIG. **2** is a perspective view, with an external housing removed to show internal features.

FIG. **3** is a front elevation view.

FIG. **4** is a rear elevation view.

FIG. **5** is a top plan view.

FIG. **6** is a left side elevation view.

FIG. **7** is a right side elevation view.

FIG. **8** is a perspective view of a rotational stroke adjustment mechanism.

DETAILED DESCRIPTION OF THE INVENTION

Organ and tissue dissociation procedures require a combination of enzymatic or chemical reagents in a solution (at a certain temperature that is optimal for the effect of the selected reagent) and mechanical forces, to enhance the tissue disaggregation and dispersion process into progressively smaller tissue fragments, clusters of cells and even single cell products.

The mechanical action is generally performed by digestion chambers that contain digestion enhancers such as marbles. The organ or tissue is placed into the selected chamber with the digestion solution (e.g., containing collagenase or specific enzyme blends). In order to mix evenly the solution and evenly increase the temperature to the desired target range, as well as to enhance the mechanical tissue dispersion within the digestion chamber, a double action (rotational and translational), variable speed and variable rate shaker is used to replace the manual movement currently utilized in several tissue processing facilities, for example to process human pancreata for the production of human islets for transplantation or research applications.

The invention has been developed to provide the necessary shaking of the chamber in a consistent and automated vertical and/or rotational manner during the isolation of beta cells from the digestion of a pancreas or of other cells from other tissues or organs. The double action (rotational and translational movement), variable speed and variable rate shaker replaces the existing shaking of the chamber by human hands which cannot provide the consistency and speed required to obtain more desirable results during the digestion of the pancreas. This shaker can perform virtually an unlimited combination of motion movements at different amplitudes and frequencies, making it an excellent machine to apply the different movements and strengths of mechanical forces that are needed during an organ and tissue dispersion process. This is particularly important because of the great variability in organ characteristics with age, conditions of the tissue and several other variables including but not limited to organ procurement, body mass composition of the donor, preservation solution, cold ischemia time before processing and the like. It is therefore critically important for the operator to be able to change the movement and the intensity of the mechanical enhancing process during tissue and organ digestion processes.

The vertical motion is controlled by a straight line Watts mechanism driven by an electric motor. The rotational movement is controlled by a separate motor connected to several linkages. Both the amplitude of the translational and rotational movement can be adjusted individually depending on the required movement of the chamber throughout the progress of the digestion of the pancreas in the chamber.

This ability to modify the amplitude, rate and combined (translational and rotational) movement of the shaker is very important even for an individual organ or tissue processing, at different times during the digestion process. For example in the case of pancreas digestion the movement is more gentle at the beginning of the process and increases in the final stages of the tissue dispersion procedure. These requirements also change when organs of different species are processed. For example, porcine islet separation from porcine pancreata requires a more gentle dissociation process compared to human pancreas processing.

The translational motion can be in any direction. In one aspect, the translational motion is a reciprocal motion in a substantially vertical plane. The reciprocal motion can be substantially vertical.

The rotational motion is imparted to the digestion chamber preferably while the digestion chamber is translating so as to provide optimal cell extraction.

Structure is preferably provided for varying the translational and rotational speed and distance. In one aspect, the translational motion is between about 1" and about 5", preferably 1.8"–4.2". The vertical oscillation frequency can be between 0 and 250 cycles per minute. The rotational motion can be between 0° and 270°. The rotational frequency can be between 0 and 60 cycles per minute. Other translational and rotational distances and frequencies are possible.

The structure for imparting translational and rotational motion to the digestion chamber can be any suitable structure. The drive mechanism can be electric, mechanical, electromechanical, pneumatic, hydraulic, or other suitable structures. Electric solenoids can be designed to impart rotational and translational motion to the digestion chamber.

An orbital shaker for cell extraction **10** is shown in FIGS. **1**–**7**. A digestion chamber **14** is provided for retaining the organ or other tissue from which cells are to be extracted. As is known in the art, the digestion chamber **14** can be provided with inlet and outlet ports for a digestive medium and other structure for facilitating the cell extraction process. A heating surface **18** can be provided for heating the medium by wrapping a supply conduit (not shown) around the heating surface **18** upstream of the connection to the digestion chamber **14**. The digestion chamber **14** is mounted an engagement arm **22** which can extend through a suitable opening **26** in the housing **30**. A chamber clamp **34** can be provided on the engagement arm **22** for engaging the digestion chamber **14**. The engagement clamp **34** permits removal of the digestion chamber **14** for servicing during the extraction process and for cleaning and sterilization.

Translational motion can be imparted by suitable structure such as motor **40**. The translational motor **40** rotates plate **44** which causes arms **48** to pivot. Arms **48** are joined to auxiliary link **52** which is pivotally mounted on shaft **56**. Pivoting of the arms **48** causes the auxiliary link **52** to pivot. Auxiliary link **52** is pivotally joined to extension link **60** at a pivot **64**. Extension link **60** is joined to drive link **68** at a pivot **72**. Drive link **68** is pivotally connected to bracket **74** at a pivot **80**. Engagement arm **22** passes through an aperture in bracket **74**. Drive link **68** is pivotally mounted on support bar **84**. A long link **88** can be pivotally mounted to support bar **84** and joined to drive link **68** by connection member **92**. Long link **88** is connected to rear bracket **76**. Engagement arm **22** passes through an aperture in rear bracket **76**.

Pivoting of the auxiliary link **52** causes reciprocating movement of the extension link **60** and drive link **68**, which in turn causes reciprocating movement of front bracket **74** and rear bracket **76**. Engagement arm **22** is thereby reciprocated in a translational movement.

The translational frequency is controlled by electronically adjusting the speed of the motor **40**. The amplitude is varied by adjusting the effective length of the arm **48**. The oscillating block **205** is attached to fly wheel **209** of motor **40**. The effective length of arm **48** is changed by the rotation of screw **204** that in turn moves the motor **40** and oscillating block **205** towards or away from the pivoting point **207** of arm **48**. This will adjust the throw of auxiliary link **52**. The screw **204** is rotated by motor **206** that is electronically controlled from the control panel.

It is sometimes preferable that the digestion chamber be subjected to rotational motion as well as translational motion. The engagement arm **22** and clamp **34** can also be rotated. This can be accomplished by suitable structure. A rotational motor **100** rotates disc **104**. A connector **108** is pivotally connected to a link **112** which is pivotally connected to a gear drive link **116**. Gear drive link **116** is connected to gear **120**. Gear **120** engages drive gear **124**. Rotation of drive gear **124** rotates pulley **128** causing belt **132** to rotate pulley **134**. Pulley **134** is joined to a shaft **138**, and rotation of the shaft **138** causes rotation of pulley **142** which is also mounted to shaft **138**. Support links **144**, **146** can be pivotally mounted between shaft **138** and support shaft **150**. A member **155** can secure support link **144** and **146** together.

Rotation of the pulley **134** under the influence of belt **132** causes rotation of pulley **142** and corresponding movement of belt **158**. Belt **158** extends to a pulley **162** that is connected to engagement arm **22**. Movement of the pulley **162** thereby causes rotation of the engagement arm **22** and engagement clamp **34**.

The rotational frequency is controlled by electronically adjusting the speed of the electric motor **100**. The rotational amplitude is changed by the rotation of arm **208**, as shown in FIG. **8**. Movement of rotating arm **208** downward from the position shown in FIG. **8** will cause link **210** to also move downward, which will reduce the stroke length. By rotating arm **208**, the pivot point of arm **210** is changed thereby changing the oscillating stroke. The rotation of arm **208** is accomplished by electric motor **212** that is controlled by the rocker switch **214** situated on the control panel **170**.

Suitable controls can be provided through a control panel **170**. The control panel **170** can have controls **174** for translational stroke length and controls **176** for rotational stroke length. Control **180** can be provided to vary translational stroke frequency and control **184** can be provided to vary the rotational frequency. Controls **188** can be provided for adjusting the temperature of the heater **18**. A display **192** can be provided to monitor temperature within the chamber **14**. It will be apparent that the translational and rotational motion can be imparted to the digestion chamber **14** through a number of different mechanisms. In another embodiment, suitable solenoids can replace the mechanical linkages and pulleys to provide translational and rotational movement of the digestion chamber.

This invention can be embodied in other forms without departing from the spirit or essential attributes thereof and, accordingly, reference should be had to the following claims rather than the foregoing specification as indicating the scope of the invention.

We claim:

1. An orbital shaker for cell extraction, comprising:

an enclosed digestion chamber having a digestion medium therein, and at least one inlet port receiving digestion medium from a supply conduit, and at least one outlet port for evacuating digestion medium from the digestion chamber;

engagement structure for engaging said digestion chamber;

translational drive structure for translating the digestion chamber;

structure for adjusting translation characteristics of said translational drive structure, said translation characteristics comprising at least one of the translation distance and the translation oscillation frequency;

rotational drive structure for rotating the digestion chamber;

structure for adjusting the rotational characteristics of said rotational drive structure, said rotational characteristics comprising at least one of the included angle of rotation and the frequency of rotation; and

said translational drive structure and said rotational drive structure being independently controllable.

2. The orbital shaker of claim **1**, wherein said translational drive structure translates said digestion chamber between about 1" and about 5".

3. The orbital shaker of claim **1**, wherein said translational drive structure oscillates said digestion chamber.

4. The orbital shaker of claim **3**, wherein said translational drive structure comprises means for oscillating said digestion chamber at an oscillation frequency of between about 0 and 250 cycles per minute.

5. The orbital shaker of claim **1**, wherein said rotational drive structure comprises means for rotating said digestion chamber through an included angle, said included angle being about 0° to about 270°.

6. The orbital shaker of claim **1**, wherein said rotational drive structure comprises means for rotating said digestion chamber at a frequency of between about 0 and 60 cycles per minute.

7. The orbital shaker of claim **1**, further comprising structure for controlling the temperature of a liquid prior to said liquid entering said digestion chamber.

8. The orbital shaker of claim **1**, further comprising clamping structure for clamping said digestion chamber.

9. The orbital shaker of claim **1**, further comprising structure for independently adjusting the translation distance, the translation oscillation frequency, the angle of rotation, and the frequency of rotation.

10. The orbital shaker of claim **1**, further comprising at least one digestion enhancer within said digestion chamber.

11. The orbital shaker of claim **10**, wherein said digestion enhancer is a marble.

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