

to a destruction of the hormone of the implanted glands by the host as a result of the incompatibility of the tissues and the subsequent reactions. The results of the present experiments only suggest that the maturity hormone among lower vertebrates is non-specific.

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**Demoor's Active Substance of the Heart.**

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Demoor<sup>1, 2</sup> has described a humoral regulatory substance elaborated by the nodal tissue of the heart which has the property of conferring rhythmicity upon the aperiodic beat of an isolated left auricle. His theory is based chiefly upon the observation that the right auricle of the rabbit or guinea pig, excised and dropped into a Ringer-Locke solution, soon assumes a regular rhythm; the left auricle, however, gives only aperiodic, jerky beats in this solution. If now, the solution in which the right auricle has been active for a while is poured over the left auricle, the latter gives rhythmical beats. Demoor has prepared both saline and alcoholic extracts of the right auricle of the rabbit which were able to confer periodicity upon the isolated left auricle of the rabbit. He has named this unknown substance the "active substance" of the heart. The writers have been able to confirm his observations.

All solutions used in the experiments were made from Baker's analyzed chemicals, recrystallized. The Ringer-Locke solution employed consisted of NaCl 0.9%, CaCl<sub>2</sub> 0.024%, KCl 0.042%, NaHCO<sub>3</sub> 0.01%, and Dextrose 0.1%. The pH was adjusted to 7.4, and the solution was oxygenated and maintained at a temperature of 37°C. throughout the experiment. An adult rabbit was placed under light ether anesthesia, the heart was exposed and perfused through the ascending vena cava with the Ringer-Locke solution for a short time to free the heart of blood. The heart was then excised and both auricles removed, care being taken to see that the venous

<sup>1</sup> Demoor, M. J., *Annales de Physiol. et de Physico-Chimie Biol.*, 1929, **5**, 1.

<sup>2</sup> Demoor, M. J., and Rylant, M. P., *Bull. Acad. Roy. de Med. de Belgique—Seance*, Oct. 26, 1929, 585.

openings were included in each preparation and that the left auricle was wholly free of septum. Each auricle was then placed in a few cc.'s of the warmed Ringer-Locke solution. In most cases the right auricle gave rhythmical beats and maintained them for varying lengths of time. In no case did the left auricle exhibit rhythmical beats although a few aperiodic beats sometimes occurred. If the right auricle continued beating for 20 to 30 minutes, pouring of the solution, in which it had been active, over the left auricle, which had remained inactive in its Ringer-Locke solution, was followed in 70% of our trials by the assumption of rhythmic beats by the left auricle. Neither histamine nor adrenalin conferred rhythmicity upon an inactive left auricle. The quiescent left auricle of the white rat also assumed rhythmicity when placed in the Ringer-Locke solution in which a right auricle of the rabbit had been allowed to beat, or when treated with a saline extract of the right auricle of the rabbit. The period during which any left auricle in this series of experiments continued beating was never over 10 minutes.

Saline extracts were made of the 2 auricles according to the following method. A rabbit was operated upon in the manner described above, except that perfusion was done with a 0.9% solution of recrystallized sodium chloride. The isolated auricles were slit open and washed free of blood by gently rubbing them with the rounded end of a fire-polished glass rod. They were then shaken free of the solution and roughly weighed. The auricles were ground to a paste with glass wool which had been boiled in redistilled water and washed repeatedly with the same. The paste was then made up to approximately 50 cc. with 0.9% sodium chloride solution, allowed to stand for 2 hours, then centrifuged at high speed for 15 minutes. The liquid was then decanted and centrifuged twice more for like periods of time. The resulting extract was again made up to 50 cc. with the sodium chloride solution. Each extract was tested for its ability to confer rhythmicity upon the inactive auricle of the white rat before subjecting it to any chemical examination. In all, 12 extracts were examined. Identical tests were conducted upon extracts of the right and left auricles. The extract of the right auricle invariably showed a higher degree of opalescence than did that of the left auricle. A definite but very thin film was always deposited upon the container of the extract of the right auricle which was absent in the extract from the left auricle. Both the substance responsible for the greater opalescence of the extract from the right auricle and this film were soluble in ether and carbon disulphide. Quantitative examination of these extracts for calcium and potas-

sium showed no difference in results for the 2 auricles. The average for calcium was 0.3 mg. per gram of tissue for the right auricle and 0.28 mg. for the left auricle. The average for potassium was 2.8 mg. for the right auricle and 2.71 mg. for the left.

Tests for nitrogen and sulphur (lead acetate reaction) were negative, as also were Nylander's test, Bial's test, Tollen's test, and the reduction of picric to picramic acid. Fehling's solution and the Shaffer-Hartman test showed slight reduction, equal in the 2 extracts. Both extracts reduced methylene blue and gave a positive test to the Molisch reaction.

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## Antigenic Structure of Certain Chlorellas and Allied Forms.

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A series of tests has been conducted in an endeavor to determine whether there is an antigenic protein fraction common to a number of related unicellular algae. The following forms have long been held in culture within our collection: *Chlorella variegata*, Chodat; *Chlorococcus humicola* (Naeg) Rabenhorst; *Mannochloris bacillaris*, Neumann; *Chlorella viscosa*, Chodat; *Chlorella vulgaris*, Beij, var. *genevensis*, Chodat; *Chlorella rubescens*, Chodat; *Chlorella luteo-viridis*, Chodat. In addition there was available another undetermined species of *Chlorella*.

Each one of the algal forms included in this list was grown upon an agar medium of the following composition: Magnesium sulphate, 0.25 gm.; calcium nitrate, 1.0 gm.; monobasic potassium phosphate, 0.25 gm.; potassium chloride, 0.12 gm.; ferric chloride, trace; distilled water, 1000 cc. This solution was now diluted threefold with distilled water and to it in turn was added 1½% agar. After sterilization, the forms were grown upon slants of the medium. Incubation proceeded in diffuse daylight at room temperature over a period of 3 weeks. The resulting growth of each organism was suspended in sterile physiological saline and then with no further treatment each antigen was injected into the posterior aural vein of a rabbit. No signs of toxicity appeared in any one of the animals as the result of this treatment. Immunization progressed by means