Biological Effects of Centrifugal Action*

A Study of the Causes of Disturbances of Equilibrium

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EIGHT years ago, being at the time engaged in studying disturbances of equilibrium consequent on lesions of the semi-circular canals, it occurred to me to subject young vertebrate animals, not yet adult, to centrifugal action in order to observe what effect this might have upon their future equilibrium.

Not being provided at that time with apparatus perfected for the object in view, I was soon forced to give up these experiments because of the great obstacle offered by the weight of the animals. I then conceived the idea of treating hen's eggs in this manner, and, in case they hatched subsequently, examining the behavior of the chicks. Two of these did hatch—and they were of minute size. Such was the curious fact which led me to take up the study of centrifugation in a systematic manner, and to record the disturbances it might engender in living matter.

I lack space to enter here into the description of the apparatus utilized in the course of my researches. I will merely give a résumé of my experiments with respect to the effects of centrifugal force in the following categories:

1. Upon solutions, salts, gels, and their passage through membranes.
2. Upon the cell.
3. Upon the sexual elements, eggs, seeds.
4. Upon nymphs.
5. Upon adult animals.
6. Upon plants.

A certain number of authors had been occupied with this question previous to myself. But while my predecessors were engaged in microscopic researches, my own have been, on the contrary, generally macroscopic, capable of being recorded by physico-chemical or physiologic methods.

To discover the effects of centrifugal force upon solutions, I performed the following experiments:

After having noted that solutions in repose during a lapse of years were no more dense in their deep-laying layers than in their superficial ones, I attempted to modify this equilibrium by centrifugation.

Cryoscopy, the calculation of densities, the investigation of superficial tensions, show that it was possible to rupture the homogeneity of solutions—this rupture, however, being very fleeting.

Hydrogels and salts of metals, on the contrary, could not be modified. I succeeded merely in expelling a few drops of water from a gel of gelatine—and this was in very special conditions.

While centrifugal force does not modify in any way the microzymases, it acts very energetically, on the contrary, upon the macrozymases.²

These results being known, it seemed advisable to me to observe how the same solutions or salts would behave in the presence of membranes.

My first attempts had in view the production of chemical tissues (of the *Leduc* type) in a centrifugator in motion. Every formula requiring a gelatine medium and a vat³ must be rejected because of the fragility of the growths considered. But it is easy to obtain "artificial plants" with a base of silicate. The products making their appearance in the apparatus are much smaller than the controls, do not attach themselves to the walls of the tubes, and present the simplest forms. From a certain number of experiments upon pseudo-cells, microbroids,⁴ one may conclude also that osmosis and diffusion under the action of centrifugal force yield manifestations which differ from those which are normal.

Osmotic phenomena are considerably increased by centrifugation, whether we make use of artificial membranes (collodion, xanthate of cellulose) or of natural membranes (peach skin, the septum of egg shells).

These few data relating to solutions, salts and mem-

branes, "taken by themselves," being ascertained, it seemed logical to study what would happen to the same elements considered in the living cell.

Doubtless the ideal thing would have been to isolate a cell.⁵ Not being able to undertake this, I utilized homogeneous tissues (tubercles of potatoes) wherein the mode of functioning is essentially the same as that of a single constituent element, apart from the idea of quantity.

My researches proved that centrifugal force draws from the cells water, sugar, ferments; that this draft is produced in a constant manner and is never complete; that it not only modifies the density of the protoplasmic juices, but that it also produces variations of a physico-chemical nature.

Thus, while the striae disappear from grains of starch taken first at the peripheral extremity⁶ of a potato, tiny x-shaped hooks become apparent on the grains of the central extremity.

While very convenient for studying the phenomena of osmosis, these tubercles lend themselves poorly to experiments concerning the nucleus and the other noble parts of the cell; therefore I had recourse to various tissues according to the cases under consideration.

The epidermis of the onion permits us to see without any difficulty the protoplasm carried away, then piled up by the centrifugation in the bottom of the cells, and the impossibility which is encountered of causing the disappearance of the Brownian movements, even by the most energetic forces.

The *Elodea Canadensis* provides an excellent field for the study of the chromoleucites, the more easy to be condensed the more hydrated the plant under examination.

Thanks to the Spirogyræ, we can easily note, according to the degree of speed employed, that the chromatophores are disturbed and then returned to position—that they can be definitely crushed—that they can be put into spheres—and that they can be finally dislocated into tiny elements very well individualized. These last, of ovoid form, more strongly colored at the two ends of their great axes, suggest chromoleucites which have been suddenly deprived of a sheath keeping them together but separated from each other.

From these few examples it is evident that centrifugal force acts upon true cells as it does upon solutions, salts and membranes. Let us see then whether it is also able to modify thoroughly systematized elements (sexual products, seeds, eggs).

In the course of my experiments I have discovered that:

1. The spermatozoa of the lower animals (*Ascaris*) support centrifugation perfectly, while those of the Mammifera are retarded in their movements.

2. Pollen, after a very long sojourn in a centrifugator, bursts in the midst of saccharine solutions, which are, however, favorable for the development of the control grains; but it is still capable of fecundating normal ovules without producing teratological types.

3. Centrifugated seeds lose weight, and their teguments become colored; and a very curious circumstance is that their germination is accelerated, retarded or arrested, according to the manner in which the centrifugation is applied.

4. The evolution of eggs is likewise hastened, made more slow or checked, and, finally, they yield a rather large percentage of parthenogenetic births.

I should like to call attention to many other details, but must confine myself to the main lines, for the further we advance in the series the more complex are the facts observed and the longer the description they require.

Before beginning the study of the phenomena produced by centrifugal force on adult animals it is advisable to examine how this force acts upon a very curious state of life: the nymphal period.

Rapid, but short, rotations produce chrysalids

⁵I do not as yet take into consideration monocellular organisms.

⁶The designation "peripheral extremity" of a centrifugated body is given to that portion of the said body which is farthest from the axis of the centrifugator, while its nearest portion is called the "central extremity."

⁷The plants issuing from seeds interrupted in their normal germination are but little modified histologically, but appear as if etiolated. Moreover they have very few absorbent hairs.

⁸Peripheral or central.

(*Bombyx*) cupped on the posterior side, whatever the orientation of the latter, letting them spout out a large quantity of liquid with respect to their weight, yet capable of living in spite of everything, and of continuing their evolution till the perfect insects appear.⁹

Slow and prolonged rotations act upon the duration of the chrysalid stage, which they either diminish or lengthen according to circumstances. I discovered also that the life of butterflies proceeding from centrifugated nymphs is shortened in proportion as the treatment is lengthened—and that their eggs, which are smaller and lighter in weight,¹⁰ yield cocoons which are themselves modified.¹¹

We now come to the disturbances sustained by adult animals through centrifugal force. While the Infusoria (green *Paramecium*, *Vorticella*), the worms (*Convoluta Roseoffensis*, *Vortex*, earthworm), the insects (chafers, *Coecinella*), undergo no alteration apparently, the fish begin to evince a certain torpor.

Beginning with the Batrachians the disturbances become clearly defined and vary with the orientation of the subject. My principal discoveries are the following:

Frogs centrifugated with "the head central" during one hour at 500 revolutions are still susceptible to violent excitations. Placed on their backs they remain there, allowing their limbs to remain as the operator places them, holding themselves in the water similarly to animals which have been curarized. Their cardiac contractions, of greatly retarded rhythm, show slight amplitude. On the contrary, subjects similarly treated, but with "the head peripheric," present gyratory motions in the direction opposite to that of the centrifugator;¹² much less influenced, they quickly return to the normal state. The beats of the heart are retarded and very ample. In both positions the teguments are red on the side where the force acts most energetically,¹³ and there exists a sanguine concentration which may proceed to the extent of the drying of tissues.

With the mammals the phenomena are still more complicated. In order to study these I oriented the animals (rabbits, rats) in five ways:

1. Head in the direction of rotation.
2. Head opposite to the rotation.
3. Head peripheric.
4. Head central.
5. Head and tail peripheric.

My observations were as follows:

1. *In the first case* (head in direction of rotation) there exists, according to the periods considered, a conjugated deviation of the head and the eyes, movements of trunk, then a hemorrhage of the cerebellum, ending in death.

2. *In the second case* (head opposite to rotation) the disturbances are nearly similar to the preceding; only the direction of the movements of trunk differs.

3. If, after having been centrifugated in the position 1 during *n* minutes, it is immediately centrifugated in position 2 for *n* minutes also, a predominance of the first lesion over the second is perceived.

4. *In the third case* (head peripheric), as in the case of the frogs, the beats of the heart are amplified and retarded; the number of red corpuscles is increased; movements "like the hand of a watch" manifest themselves; death occurs through sanguine inundation of the metencephalon.

5. *In the fourth case* (head central) the subjects observed drag their posterior quarters, and upon autopsy exhibit a brain of waxy aspect.

6. *In the fifth case* (head and tail peripheric) the lesions are not, as one might believe *a priori*, the grouping of those obtained in the positions 3 and 4. There exists a conjugated deviation of the head and the eyes, a rocking or nodding of the head, an anes-

⁹These insects are stunted and are poor procreators.

¹⁰The differences of size and weight are much more perceptible when we examine the eggs born of parents centrifugated at the nymphal period for several generations.

¹¹Those cocoons, which are smaller and shorter, are spun on the bare ground, and yield a lower percentage of females than of males. The evolution of the latter is more rapid than that of the former.

¹²These movements have nothing in common with the theory of "anti-kinesis." Cf. Raphael Dubois: *The Natural Origins of the War*. Georg, Lyons, 1916.

¹³This color is due simply to the dilatation of the capillaries.

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¹The ideas sketched in this article are developed in my *Contribution to the Study of Experimental Centrifugation in Biology*. (Thesis Doctor of Natural Sciences, published by Rey, 4 Rue Gentil, Lyons, 1917).

²As types of macrozymases we selected the luciferase and the purpurase of R. Dubois.

³For example: pellets of sulphate of copper and sugar immersed in a gelatinous solution of ferrocyanide of potassium.

⁴R. Dubois: *Mineral Cultures: Radiobes and Eobes*. C. R. Congrès de Liège, 1905.

thesia of the posterior quarters, and upon autopsy a slight congestion of the encephalon.¹⁴

I will make no report upon my timid attempts at human centrifugation, too incomplete as yet on account of the war,¹⁵ but will point out, as briefly as possible, the alterations produced by centrifugal force in adult plants.

Suitably applied, this force modifies: the external aspect of a culture of Thallophtyes, the size of the constituent elements and the activity of certain secreted products.

It is possible to sterilize sharp wines and damaged beers by simple centrifugation.

Finally, there is a very general idea which becomes evident from my investigations. This is that the more vigorous an organism is the denser it is. Thus a microbe, a diseased yeast are, for equal size, less heavy than a microbe or a yeast enjoying full physiologic activity.

With respect to experiments upon the superior plants it was found that the lots oriented with "peripheric roots" present a marked enlargement of the subterranean system as compared with the aerial portions, while those disposed with "central roots" cease to be subject to the action of geotropism, which is gradually annulled by the centrifugal force, as has been shown by Knight.

The ensemble of my investigations proves that the study of the effects produced by centrifugation belongs essentially to the domain of general physiology since in a number of cases these effects are comparable in both plants and animals.

We have seen that the centrifugal force violates the normal cellular equilibrium by activating the osmotic exchanges and by modifying the concentration of the juices; that it is capable of accelerating, retarding or preventing the development of eggs and seeds, of changing the weight and the size of certain organisms, and of occasioning disturbances capable of producing in living creatures internal or external anomalies.

But what is the reason for the manifestation of so many modifications? Is it because of simple pressure? Not only does a pressure exerted by other processes fail to give the same results, but the subjects thus treated will be destroyed. Besides the very special sort of pressure secured by the centrifugators another factor intervenes. Everything leads us to believe that we are here concerned with a case of dehydration, of drying of the tissues. In fact:

1. Organisms which are but slightly hydrated are not attacked by centrifugal force (the chafer, *coccinella*); those containing, on the contrary, a large percentage of water, are the ones most modified (*Vertebrata*).

2. Aquatic creatures centrifugated in their own element are not at all, or but little disturbed (*Vorticella*, *Paramecium*).

3. The butterflies of the *Bombyx* cannot pierce their cocoons in default of liquid to displace the threads.

4. The hyperconcentration of the blood, the increase in the number of red corpuscles, indicate the diminution of humor.

5. When sufficiently prolonged centrifugation desiccates frogs even more completely than the saline solutions employed by Claude Bernard.

6. The admirable investigations of Raphael Dubois long ago demonstrated the dehydrating action of general anesthetics. But in a series of experiments I have obtained a concordant between the results obtained by centrifugal force and by anesthetic vapors.

From all these facts it seems, therefore, to result that disturbances consequent on the centrifugation of living beings are due to two causes: the dehydration of the tissues on the one hand, and their compression (under a very special form) on the other.

State-Aided Research and the Small Manufacturer

SINCE the war started every one of the allied nations has come to realize how much they depended on Germany for a great number of manufactured articles. Because Germany sold these things at a low price, made possible by their syndicates and government assisted associations, we were willing to let them monopolize the business. This was further made possible by the clever propaganda, inaugurated years ago, for the manipulation of import laws and duties in such a way as to preclude the possibility of com-

¹⁴Of course we observe, besides the disturbances noted, a whole series varying with the degrees of speed employed and the length of time. The animals return to their normal state unless there is a systematic endeavor to produce death.

¹⁵The apparatus employed, being of German origin, was sequestered at the beginning of the mobilization.

petition by manufacturers in other countries. The history of dye stuff tariffs in the United States is an excellent example of German intrigue of this nature. Now these monumental blunders are being realized, and strenuous efforts are being made in every country to win commercial freedom, at the same time that our armies are fighting for political freedom.

In England systematic efforts are being made in this direction, and a report of the British Committee for Scientific and Industrial Research, extracts of which are given below, as published in the *Journal of the Society of Chemical Industry*, should be of wide interest in the United States:

Although adequate provision has been made for the prosecution of investigations of pressing national concern, such as those on fuel, timber and concrete, the spade-work of the past year has been largely concentrated on the organization of cooperative industrial research, for which purpose there has been established a fund of £1,000,000 to be expended over a period of five or six years as grants in aid to Research Associations, the maximum grant to any one association not to exceed the total of the contributions of its constituent firms.

In addition to the two classes of research above mentioned, the national and the cooperative, there is a third class which covers the work of individuals and of independent manufacturers; and it is in respect of the last-named that criticisms and complaints have begun to appear. No definite or satisfactory scheme, it is said, has been devised for helping the small, independent manufacturer by means of monetary grants, or by relief from taxation; and in many cases, the incidence of the excess profits tax has fallen upon him with such severity that research work has had to be postponed or abandoned. The larger firms which have joined a Research Association have been allowed their contributions to it as a working expense for the purposes of income tax, excess profits and war profits taxes: why should not the same privilege be allowed the manufacturer who prefers to "gang his ain gait," or is compelled thereto, owing to lack of facilities for cooperation? Surely the State does not wish to cripple the independence or initiative of the individual manufacturers to which British industry has owed so much in the past?

Before proceeding to discuss these claims, it would be well to consider first what the committee has to say upon the subject. On the general question of cooperative *versus* individual action, the report is very definite. "It is intended that the Research Associations should include as many of the firms in each industry as possible" (p. 12). Notwithstanding the fact that under the heading "Researches unsuited for Co-operative Action" only far-reaching problems of national importance are specified (p. 15), we read on the next page that there is room for private research by individual firms on the lines of their own special work, and they may gather many suggestions for this from the results of the cooperative investigations. No attempt is made to distinguish between work best fitted to be performed inside the works, and that to be done outside a subject—of no small importance. Research by the individual firm is further referred to as being undertaken "at its own expense."

Reference to the position of firms which do not participate in the new scheme of cooperative research is to be found in two paragraphs at the end of the report (pp. 44 and 45). The cooperative associations are expected to be very useful to their constituent firms in helping them to solve difficulties encountered in the course of manufacture, "but it will take time for all the many industries of the country to organize themselves, and meantime, we shall do our best to assist inquirers to obtain the best advice available." In those cases where the problem has a *prima facie* connection with research, the Royal Society will assist the committee in selecting an institution, or research workers, to carry out the investigation. In suitable cases, the committee will formulate the problem to be investigated, and will pay for the services of an assistant if required. If the work proves successful, the manufacturer will be asked to pay for either the whole or a part of the expenses incurred, and in return he will have the exclusive benefits of the research "for a period to be agreed upon." An alternative method of assistance is also foreshadowed. A "young" research worker may be attached to the works laboratory for a definite period, during the first term of which the department would pay the whole of his salary, during the second period it would pay a moiety, and during the third and last the manufacturer would bear the whole expense.

There is thus, apparently, no intention of assisting the unattached manufacturer by means of grants in aid, nor of helping him by allowing the costs of research to be paid for out of excess profits; but the general tenor of the report, and the brevity of the allusions to this part of the subject, lead to the inference that the question is still under consideration, and that the committee is open to revise any of the tentative conclusions it has arrived at.

Although no kind of combination between manufacturers other than that for the purpose of research is mentioned or implied, the small manufacturer who looks with repugnance upon the development of cartels, trusts and other forms of combination will probably see in this advocacy of cooperative research merely the thin end of a wedge which, if driven further, would lead to his ultimate extinction as an independent unit. He might further argue that the facilities for cooperation in research possessed by the larger firms, such as those engaged in the "heavy" chemical industry where fusions and "understandings" have been multiplying of late, are undoubtedly greater than those which are at present available to him, and that, pending the solution of the very difficult problem of organizing the small manufacturers, he should at least receive an equal measure of State recognition. The carrying out of his researches at an institution, probably remote from his works and over which he would have but little control, or the attachment to his laboratory of a research worker, who, being specified as "young," would probably lack experience, would not satisfactorily meet his needs; and even if he were permitted to allocate the costs of research to working expenses for the purposes of taxation, he would still be at a disadvantage compared with his "combined" rival, inasmuch as he would not have at his disposal the well-equipped organization of a cooperative association. To the unattached manufacturer, who has either been unable to initiate research work, or has been forced to abandon it from pecuniary considerations, the retort might be made that if he had invested in research before the war the cost of it could now be included in his working expenses, and that his present dilemma is but a consequence of his previous neglect or want of foresight; such an argument, however, would come badly from a State department, whose primary object is to encourage research, here and now. Nor does the contention that the revenue must be protected appear to carry any great weight, for much of the research work in question would have some national importance, and judged with reference to current expenditure, the sum involved would be very small.

In answer to the objection that if chemical manufacturers were assisted in the manner described a multitude of other manufacturers and traders would present similar demands, the reply could be made that each application should be considered on its merits, the main criterion being that the work for which aid was sought should be of distinct national importance. Assuming now that the principle of direct financial assistance were ceded, it would yet remain to devise a workable scheme of applying it. The report seems to suggest that the small manufacturers should join existing associations, but it is obvious that in many cases this would not be possible, and it would therefore be necessary to form new associations. The organization of these would be a formidable task, as the manufacturers themselves would be too fully occupied to devote the necessary time to it, and the selection of properly qualified State officials would be no less difficult, even if the officials as such were acceptable to the manufacturers. An alternative plan would be to allow a certain percentage of the excess profits to be expended upon research, the magnitude of which would vary inversely with the pre-war standard of profits, the maximum percentage being allowed to such firms which had no pre-war standard at all. A third method would be to make direct grants in aid, to vary in amount according to the needs of the individual firms. Such a proposal would involve a large amount of investigation work on the part of the department, and it is doubtful if the manufacturer would be willing to submit to such an ordeal.

The second plan seems least open to objection, but the whole question is beset with difficulties and needs very careful consideration. From a cursory survey of the arguments for and against, we incline to the view that the small independent manufacturer has a good case for greater assistance than that promised or adumbrated in the report; at the same time, the underlying principle of the committee's proposals—cooperation for the common weal—admits of no refutation.