THE EFFECT OF THE INTERNAL SECRETIONS UPON THE DIVISION ENERGY OF PARAMOECIA

G. L. ROHDENBURG

(Under a grant from the Frederick S. Winegarten Fund)

The basic problem in the genesis of cancer is a knowledge of the various factors which influence the division energy of cells. Although the term division energy is frequently used synomonously with growth energy, it is a distinctly different phase of cellular biology. There are so many factors concerned in the process of cell division in a multicellular organism, that it is quite impossible to assign to each factor its proper sphere of influence. In consequence investigations of this problem are best confined to single celled organisms such as the paramoecia, where the multiplicity of factors may be considerably reduced and the fundamental ones therefore more readily recognized.

The influence of changes in the electrolyte content of the food upon the division rate of paramoecia has been studied by Packard (1) while in another report we (2) have shown the influence (upon this cell power) of the physical state of the medium in which the organisms are grown. Others, among whom is Hammet, have shown the influence of specific chemical compounds. The present report presents the result of a study of the various specific or supposedly specific products of glands of internal secretion upon the division rate of these organisms.

A standard medium was prepared as described by Packard (1) and to this one or another of the internal secretory products was added in varying strengths as indicated in the protocols. With each series of treated paramoecia, a line of non-treated was carried along, in order to control the influence of temperature, and other at present unrecognized factors. In each group, six lines were carried for two periods of six days each.

Adrenal Gland. Three products of this gland were employed. One, adrenal hydrochloride, was added to the stock medium in

the proportion of one drop of a 1 to 1,000 solution to 10 drops of medium. The treated group divided 87 times in 12 days, giving a line average of 14.5. The controls during this same period divided 60 times giving a line average of 10. In the treated group the division rate was 45 per cent higher than the controls.

In a second series, powdered adrenal cortex was used. One gram of the powder was placed in 100 cc. of distilled water and a few crystals of chlorotone were added. After thorough shaking, the mixture was allowed to stand for 24 hours and then filtered. Chlorotone was added to the filtrate. One drop of the filtrate was added to 10 drops of medium. During the period of observation the treated group divided 154 times giving a line average of 25.6, while the controls divided 71 times giving a line average of 11.8. The division rate of the treated group was 117 per cent higher than the controls.

In a third series, a commercial extract of the adrenal gland cortex (Harrower) was employed (the manufacturer states that 1 cc. equals five grams of fresh cortex). One drop of this extract was added to 10 drops of medium. The treated group showed 382 divisions during the period of observation as compared with 243 divisions in the controls. The line averages were respectively 63.6 and 40.5. The treated group showed a stimulation of 57 per cent.

Disregarding exact percentages, since statements based on them are fallacious unless founded upon thousands of observations, one may conclude that the products of the adrenal gland stimulate the division energy of paramoecia, and that the stimulatory principle is probably present in largest amount in the cortex.

Pancreas. While the pancreas has several secretions, only one, Insulin, was employed. One drop of Insulin (20 units per cc.) was added to 10 drops of medium. The number of divisions in the treated group for the 12 day period was 35, the controls dividing 60 times in the same period. The line averages were 5.8 and 10 respectively. In this group, Insulin depressed the division rate 42 per cent.

Parathyroid. The parathyroid hormone (Collip, 20 units per

cc.) was used. The dilution was one drop of hormone to 10 drops of medium. The treated lines divided 163 times giving a line average of 27.1 while the controls divided 544 times giving a line average of 90.6. The parathyroid hormone depressed the division rate 70 per cent.

Thyroid. A 1 to 1,000 solution of Thyroxin in distilled water was prepared and one drop of this was added to 10 drops of medium. During the 12 day period the treated group divided 60 times giving a line average of 10 while the controls divided 100 times giving a line average of 16.6. Thyroxin depressed the division rate 39 per cent.

Ovary. A commercial extract (Burroughs & Welcome) of whole ovary was used in the first group. One drop of this was added to 10 drops of medium. During the period of observation the treated group divided 221 times giving a line average of 36.8 while the controls divided 251 times giving a line average of 41.8. The change in the division energy is not significant.

A second series was conducted using a commercial preparation (Agomensin). This was employed, one drop of the reagent to 10 drops of media. The treated line divided 290 times giving a line average of 48.3 while the controls divided 281 times giving a line average of 46.8. The change in the division rate is not significant.

A third series was carried using a corpus luteum extract in dilution of 1 to 50 but the colloidal suspension resulting from this mixture completely inhibited all cell division in the treated group. The experiment was therefore discontinued.

One may conclude that the ovary has no influence on the division rate.

Spleen. One gram of dried spleen substance was placed in 100 cc. of distilled water and thoroughly shaken, chlorotone was added. After 24 hours the mixture was filtered and one drop of the filtrate was added to 10 drops of medium. In 12 days, the treated series divided 116 times giving a line average of 19.3. During this same period the controls divided 44 times giving a line average of 7.3. The treated group showed a stimulation of division energy of 164 per cent.

Liver. A number of different liver preparations were employed. All of the preparations were prepared as follows: to 100 cc. of distilled water one gram of the dried powder was added together with some chlorotone. After thorough agitation the mixtures were allowed to stand for 24 hours and then filtered. One drop of the filtrate was added to 50 drops of medium. The preparations used were Dried Liver (two brands), Liver Extract having a haemopoietic activity, Anabolin, a liver extract supposed to depress the blood pressure. The data of the various lines is appended in table form.

TABLE I

Substance	Divisions in Treated	Line Average	Divisions in Controls	Line Average
Liver dried (*a*)	108	18	44	7.2
Liver dried (*b*)	141	23.5	104	17.3
Liver extract	199	33.1	104	17.3
Anabolin	234	39	104	17.3

It appears from these experiments that liver stimulates the division rate to varying degrees: Dried Liver (*a*) 63 per cent, Dried Liver (*b*) 32 per cent, Liver Extract 91 per cent, Anabolin 125 per cent.

Testes. Dried orchitic substance was used, one gram being suspended in 100 cc. of distilled water with the addition of chlorotone crystals as a preservative. After allowing the suspension to stand for 24 hours it was filtered and used, one drop of the extract to 10 drops of medium. The treated series divided 84 times, the line average being 14, while the control group divided 39 times, a line average of 6.5. Orchitic extract stimulated the division energy 115 per cent.

In a second group, a commercial orchitic tablet (Burroughs & Welcome) was used. The tablet was reduced to a powder and the powder was treated as was that of the first group in this series. One drop of the extract was added to 10 drops of media. The control group divided 754 times giving a line average of 125.6 while the treated group divided 771 times giving a line average of 128.5. The effect on the division rate is negligible.

Thymus. Three preparations were used. The first was the dried gland itself. This was prepared as were the other tissues and used in a dilution of 1 to 10. After 12 days observation it was found that the treated group had divided 61 times giving a line average of 10.1 while the control group had divided 39 times giving a line average of 6.5. The stimulation of the division energy was 55 per cent.

In the next series a hormone was prepared after the method of Collip, with the parathyroid gland. This hormone will be described elsewhere. With this product the treated group divided 171 times giving a line average of 28.5 as compared with 39 divisions in the control group, a line average of 6.5. The division rate was stimulated 338 per cent.

A third series was treated with a hormone (?) containing a large amount of lipoid. This when mixed with the medium, made a heavy colloidal suspension which, as has been shown elsewhere, inhibited all division. The experiments were therefore discontinued.

Pituitary. In the first series, dried entire pituitary gland was used. This was prepared as were the other dried glands and used, one drop of the filtrate to 10 drops of medium. During the period of observation the treated lines divided 122 times giving a line average of 20.3. The controls divided 296 times giving a line average of 49.3. The division rate was inhibited 59 per cent.

In a second series surgical pituitrin was employed, one drop of the pituitrin being added to 100 drops of media. During the observed period the treated lines had 232 divisions, giving a line average of 43.6, while the controls had 544 divisions with a line average of 90.6. The inhibition of the division rate was 51 per cent.

In a third series the dried, powdered posterior lobe was used, the filtrate being prepared as before. It was used, one drop of the filtrate to 10 of medium. In the treated group there were 811 divisions, giving a line average of 135.1. During the same period, the controls divided 754 times giving a line average of 125.6. The stimulation of the division rate was less than 10 per cent and is not significant.

Breast. In the final series dried breast tissue (of course of doubtful endocrine influence) was prepared as were the other dried products. It was used, one drop of filtrate to 10 drops of media. The control group divided 754 times giving a line average of 125.6. The treated group divided 1055 times giving a line average of 175.8. The stimulation of division energy was 39 per cent.

Discussion

Our results may be classified in one of three groups, stimulation, retardation, or no effect.

Are these effects due to some specific property of the glands or to extrinsic forces? With all of the extracts chlorotone was used as a preservative. The effect of this agent upon the division energy can be discounted since three different effects are present. It is not likely that a given substance using the same dose would at one time stimulate and at another depress. The influences of medium and room temperature, are controlled by the untreated groups. The hydrogen ion of the media was not influenced by the addition of the material tested. The physical state of the menstrum was also controlled; and mixtures which were of colloidal character were discarded since it has been shown that this state of the medium depresses the division energy. By elimination, the results must be attributed to specific properties shared in common by several different histological types of tissue, and possibly independent of any other specific action of such tissue secretions.

A mathematical statement as to the relative degree of stimulation is unsafe on the basis of the number of observations recorded. Subject to this reservation, the order of stimulation was spleen, liver, thymus hormone, adrenal cortex, testes, adrenal hydrochloride. Inhibition was least evident in thyroxin, increasing progressively with insulin, entire pituitary gland, surgical pituitrin, parathyroid.

The interesting speculative idea develops that in the tissues of complex organisms, such as the vertebrates, there are two forces, one which inhibits cell division, another which stimulates. It seems possible that both stimulator and inhibitor are capable of

chemical purification and concentration. It would be of interest to study the effect of stimulators upon the genesis of neoplasia in foci of chronic irritation; and of inhibitors upon the rate of growth of transplanted neoplasms. The further speculative idea is suggested that since splenic extract is a stimulator, one of the functions of the leucocytes in wound healing may be to bring to a given site substances which stimulate wound healing.

Conclusions

The actions of the various endocrine glands upon the division energy of paramoecia varies:

Spleen, liver, thymus, adrenal and testes stimulate.

Ovary has no influence.

Thyroxin, insulin, pituitary and parathyroid inhibit.

RÉFERENCES

(1) PACKARD, CHARLES: The effect of sodium on the rate of cell division. J. Cancer Res., 1926, x, 1.

(2) ROHDENBURG, G. L.: Colloids as regulators of the division energy of cells. J. Cancer Res., 1929, xiii, 242.