QUOTATIONS

MEDICAL RESEARCH

MEDICAL research is of so continuous a nature that attempts to review its progress at any given time are necessarily fraught with great difficulty. Observations and experiments which to-day assume large proportions may to-morrow seem relatively insignificant in the light of clearer understanding. On the other hand, labors which at this present hour appear to hold out but little promise of immediate utility may come to occupy a first place in the records of achievement. These facts should be borne in mind in considering the annual report of the Medical Research Council, a summary of which appears in another column. The number of investigators initiated or assisted by the council during the past year is so great as to be almost bewildering. These investigations, too, cover a field within the limits of which are included many other sciences distinct from medicine, yet, as is now known, ancillary to it. If certain observations appear to stand out from the others, and in consequence attract a larger share of attention, this fact must not be allowed to discount the possibility that the more obscure may yet prove to be the more important work. That proviso having been made, it may be acknowledged that the researches of the past year have been fruitful. New light has been obtained on some of the more recondite processes of nutrition. The importance of sunlight in the early years of life has found substantial and striking confirmation in the study of rickets carried on at the Kinderklinik in Vienna by workers from the Lister Institute and the Medical Research Council. Further, the discovery made at Toronto of a method of obtaining the anti-diabetic substance "insulin" seems likely to effect great changes in the treatment of this disease. That discovery belongs in no sense to the Medical Research Council, which merely recounts its history and pays tribute to its value. Nor, as our correspondent shows, have other scientific bodies been idle during the period under review. The papers submitted last night to the Royal Society of Tropical Medicine and Hygiene on the treatment of African sleeping sickness, if they do not profess finality, at least advance this difficult problem a stage farther towards solution. The German drug known as "Bayer 205," which was introduced to professional attention some short time ago, has afforded in the hands of British tropical disease investigators a large measure of success. Its failures are not less interesting or instructive; nor is the parallel between it and the preparation of arsenic, elaborated at the Rockefeller Institute and recently subjected to trial in Africa, without its significance. These observations, like the observations made at home and in temperate areas of the empire, have abiding value, whether or not the conclusions based on them remain valid. For they represent conscientious and disinterested work undertaken with no other idea than the advancement of knowledge. This quality of disinterestedness is the noblest possession of science. So long as it is maintained unsullied the faith which millions to-day repose in the exercise of human reason as the cure of human ills will not fail of justification.—*The London Times.*

SPECIAL ARTICLES

THE MINIMUM CONCENTRATION OF LUCIFERIN TO GIVE A VISIBLE LUMINESCENCE

LUCIFERIN is the substance of luminous animals which produces light when oxidized. An enzyme, luciferase, likewise found in luminous animals, must be present with luciferin for light production. In 1916[1] I calculated that one part of luciferin from an ostracod crustacean, *Cypridina,* in 1,700,000,000 parts of sea water, when mixed with luciferase, gave a light visible to the unaided eye. This figure is so much smaller than the concentration of substances detectible by ordinary chemical reactions, which at most run into the millions, that I have repeated these experiments, only to find that my preceding estimate may be bettered. The light from weaker solutions of luciferin than 1:1,700,000,000 can be seen.

In this work an important source of error has appeared which must be carefully guarded against and to which I call special attention below. The exact procedure of determining the minimum concentration is as follows: 0.0954 grams of dried *Cypridinae* are weighed out, ground in a mortar, dissolved in boiling[2] sea water and quickly diluted to 954cc cold sea

1 *Amer. Jour. Physiol.,* XLII, 335, 1917.

2 Boiling destroys the luciferase, but not the luciferin.

water.[3] When filtered this forms the stock solution which contains one part dry *Cypridina* in 10,000 parts sea water. One part stock solution to 99 parts sea water gives solution A, that is, 1:1,000,000 solution, and one part of solution A to 99 parts sea water gives solution B, that is, 1:100,000,000 solution. If A is mixed with an equal volume of luciferase solution,[4] quite a bright light results. If B is mixed with an equal volume of luciferase solution,[4] a faint light results. B/2 gives a very faint light and B/4 gives a light so faint that the fully dark adapted eye can hardly detect it. Some sets of observations were made by myself and some by Mr. K. P. Stevens. We remained in a dark room for at least three quarters of an hour in order to increase the sensitivity of our eyes. In mixing the solutions, 5 c.c. of luciferase was poured into 5 c.c. luciferin in a test tube, 18 mm. in diameter. If we assume that B/2 is the solution giving light just detectable with the eye, note that its concentration is halved on mixing with an equal volume of luciferase, so that we can without doubt see the light from a B/4 or a 1:400,000,000 solution of the substances in dry *Cypridina*.

The remainder of the calculation depends on the per cent. of luciferin in dry *Cypridina*. It must surely be less than 10 per cent. and, judging by the size of the luminous gland, greater than one per cent. We can, therefore, safely say that one part of pure luciferin in between 4 and 40 billion parts of sea water gives, upon oxidation, light visible to the unaided eye.

A similar experiment with luciferase shows that one part of pure luciferase in between 800 million and 8 billion parts of sea water will oxidize a stock luciferin solution with visible luminescence.

In the above work new glassware that had never been used for luciferin, or in fact for any other purpose, was employed. This is necessary as can be seen from the following experiment. A 100 c.c. graduate cylinder which had held the stock solution of luciferin (1 part dry *Cypridina* to 10,000 sea water) was washed in fifteen changes of hot water (not too hot for the hands). The graduate cylinder was then filled with sea water which stood in the cylinder for two minutes and was then tested with luciferase. A very faint light appeared. After standing five minutes the light from the sea water in the cylinder mixed with luciferase was brighter and after standing ten minutes quite a fair light was produced. Sea water which had not stood in this graduate cylinder gave no light with luciferase.

Evidently luciferin adsorbed on the walls of the cylinder is slowly liberated into the sea water. It is not removed by mere washing in fifteen changes of hot tap water. If I had used this particular graduate, supposedly clean, to hold any of my dilute solutions of luciferin it is obvious that serious error would have appeared in determining the minimum concentration of luciferin for luminescence.

Adsorption is no new phenomenon. We allow for it in dealing with the large surface areas of powders but generally overlook it on the surface of measuring vessels. However, adsorption is not to be neglected in an experiment dealing with parts per billion. The adsorbed body is especially likely to come off if the vessel is used with another solvent. Dye on the surface of glass that will not come off on washing in water may be removed by washing in alcohol or in slightly alkaline or slightly acid water.

I would suggest that the extraordinarily small concentration of Botulinus toxin necessary to kill mice (3×10^{-18} c.c. regularly and 3×10^{-21} c.c. sometimes) as reported by Bronfenbrenner and Schlesinger[5] may possibly be explained by "seepage" of adsorbed toxin from vessel walls in some such way as I have just indicated. One can not be more specific in pointing out any error in this work as the method of dilution of the crude toxin is not described in detail. A concentration of 10^{-21} c.c., however, requires careful scrutiny.

It is interesting to compare the minimum concentration for luciferin luminescence with the concentration of colored solutions at which the color is just perceptible. Mr. Stevens has made some determinations with dyes, such as methy-

[3] Rapidity in making dilution is essential to reduce spontaneous oxidation of luciferin to a minimum.

[4] 0.1 gram dry *Cypridina* to 100 c.c. cold sea water allowed to stand until luminescence ceases and filtered.

[5] *Jour. Am. Med. Assoc.*, 78, 1519, 1922; SCIENCE, LVI, 280, 1922; cf. Stehle, SCIENCE, LVI, 143, 1922.

lene blue, acid fuchsine, gentian violet, methylene green, etc., in 18 mm. test tubes observed in the daylight from the side, and finds the limit of color visibility in the ten millions, one thousand times above the limit for luciferin luminescence. There is one exception, which concerns not transmitted light, but fluorescence. A faint greenish fluorescence of fluoresceine is clearly visible in the light from a powerful carbon arc focussed in the solution, with one part of the dye in 10 billion parts of ordinary distilled water. Such water is not by any means optically empty and light scattering from dust particles interferes with the observation of fluorescence. Spring[6] has reported that with optically empty water, the fluorescence from 10^{-15} grams per c.c. of fluoresceine can be detected in the beam of an arc lamp.

Thus, luminescences from very small quantities of matter can be detected. We have an extreme case in the flash of light from impact of a single charged helium atom on fluorescent zinc sulphide. Biological reactions can not approach this in sensitivity, but I believe the value for *Cypridina* luciferin mentioned above sets a new record for chemiluminescence.

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X-RAYS AND THE SEX CHROMOSOMES

IN two previous issues of this journal[1] the writer has reported what appeared to be the production of non-disjunction of the sex chromosomes of Drosophila by X-rays. When primary non-disjunction is found, as happens rarely, in ordinary cultures the two kinds of exceptional offspring do not occur in equal numbers. The exceptional sons which arise from the fermentation of eggs containing no X-chromosome by sperm containing an X-chromosome are considerably more numerous than the exceptional daughters which arise from the fertilization of eggs containing two X-chromosomes. Since the effect of X-rays is to increase proportionately the numbers of exceptional sons and daughters, the increased number of exceptional daughters obtained as a result of X-raying their mothers is considerably less than the increased number of exceptional sons. Up to the time of publication of the notes to which reference is made above, the data obtained showed clearly that X-rays caused an increased production of exceptional sons and probably also of exceptional daughters. Since that time the technique[2] has been improved and a large number of experiments have been performed. The data now accumulated make it reasonably certain that X-rays not only cause non-disjunction but that the exceptional daughters of X-rayed mothers are in most cases fertile and themselves produce exceptional offspring. Reference will here be confined to the third and fourth series of experiments which were performed with the improved technique. The results of the third series were stated at the Boston meeting of the American Association. The fourth series of experiments have only recently been completed.

The improvements in technique involved, among other things, using for the X-rayed mothers virgin heterozygous flies resulting from crossing white-eyed long-winged females with eosin-eyed, miniature-winged males. This made it possible to test for the presence of exceptional females among the flies to be X-rayed. After X-raying, the females were mated to wild-type males. The use for X-raying of females heterozygous for characters located in the sex chromosomes made it possible to follow individually by the characters of the offspring the sex chromosomes which had been submitted to X-rays. In the third series of experiments 76 females were X-rayed and 79 females were kept as controls. In each experiment the X-rayed and control females were sisters. The X-rayed mothers produced a total of 1557 regular sons, 42 exceptional sons, 1771 regular daughters and 8 exceptional daughters. Seven out of eight exceptional daughters were fertile

[6] Acad. Roy. de Belg. *Bull.* Class. sc., 1905, p. 201-211.

[1] "On the elimination of the X-chromosome from the egg of Drosophila melanogaster by X-rays," SCIENCE, Vol. LIV, pages 277-279, September 23, 1921; "The production of non-disjunction by X-rays," SCIENCE, Vol. LV, pages 295-297.

[2] The technique of these experiments is described in detail in a paper submitted to the editors of the *Journal for Experimental Zoology.*