

ported, several different dosages were made use of, and while the figures are not yet quite conclusive they make it probable that, within the limits used, the number of recessive lethals does not vary directly with the X-ray energy absorbed, but more nearly with the square root of the latter. Should this lack of exact proportionality be confirmed, then, as Dr. Irving Langmuir has pointed out to me, we should have to conclude that these mutations are not caused directly by single quanta of X-ray energy that happen to be absorbed at some critical spot. If the transmuting effect were thus relatively indirect there would be a greater likelihood of its being influenceable by other physico-chemical agencies as well, but our problems would tend to become more complicated. There is, however, some danger in using the total of lethal mutations produced by X-rays as an index of gene mutations occurring in single loci, for some lethals, involving changes in crossover frequency, are probably associated with rearrangements of chromosome regions, and such changes would be much less likely than "point mutations" to depend on single quanta. A re-examination of the effect of different dosages must therefore be carried out, in which the different types of mutations are clearly distinguished from one another. When this question is settled, for a wide range of dosages and developmental stages, we shall also be in a position to decide whether or not the minute amounts of gamma radiation present in nature cause the ordinary mutations which occur in wild and in cultivated organisms in the absence of artificially administered X-ray treatment.

As a beginning in the study of the effect of varying other conditions, upon the frequency of the mutations produced by X-rays, a comparison has been made between the mutation frequencies following the raying of sperm in the male and in the female receptacles, and from germ cells that were in different portions of the male genital system at the time of raying. No decisive differences have been observed. It is found, in addition, that aging the sperm after treatment, before fertilization, causes no noticeable alteration in the frequency of detectable mutations. Therefore the death rate of the mutant sperm is no higher than that of the unaffected ones; moreover, the mutations can not be regarded as secondary effects of any semi-lethal physiological changes which might be supposed to have occurred more intensely in some ("more highly susceptible") spermatozoa than in others.

Despite the "negative results" just mentioned, however, it is already certain that differences in X-ray influences, by themselves, are not sufficient to account for all variations in mutation frequency, for the present X-ray work comes on the heels of the determination of mutation rate being dependent upon tempera-

ture (work as yet unpublished). This relation had first been made probable by work of Altenburg and the writer in 1918, but was not finally established until the completion of some experiments in 1926. These gave the first definite evidence that gene mutation may be to any extent controllable, but the magnitude of the heat effect, being similar to that found for chemical reactions in general, is too small, in connection with the almost imperceptible "natural" mutation rate, for it, by itself, to provide a powerful tool in the mutation study. The result, however, is enough to indicate that various factors besides X-rays probably do affect the composition of the gene, and that the measurement of their effects, at least when in combination with X-rays, will be practicable. Thus we may hope that problems of the composition and behavior of the gene can shortly be approached from various new angles, and new handles found for their investigation, so that it will be legitimate to speak of the subject of "gene physiology," at least, if not of gene physics and chemistry.

In conclusion, the attention of those working along classical genetic lines may be drawn to the opportunity, afforded them by the use of X-rays, of creating in their chosen organisms a series of artificial races for use in the study of genetic and "phaenogenetic" phenomena. If, as seems likely on general considerations, the effect is common to most organisms, it should be possible to produce, "to order," enough mutations to furnish respectable genetic maps, in their selected species, and, by the use of the mapped genes, to analyze the aberrant chromosome phenomena simultaneously obtained. Similarly, for the practical breeder, it is hoped that the method will ultimately prove useful. The time is not ripe to discuss here such possibilities with reference to the human species.

The writer takes pleasure in acknowledging his sincere appreciation of the cooperation of Dr. Dalton Richardson, Roentgenologist, of Austin, Texas, in the work of administering the X-ray treatments.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN INSTRUMENT FOR REPEATED DETERMINATIONS OF BLOOD VISCOSITY IN AN ANIMAL¹

In experiments where it is desirable to make repeated determinations of the viscosity of the blood of an animal, the withdrawal of the amount of blood

¹ From the Physiological Laboratories of the University of Chicago and the University of Western Ontario, London, Canada.

for the determinations and its replacement by fluid of less viscosity from the tissues causes a progressive diminution in the viscosity of the blood.

In order to make viscosity determinations without withdrawal of blood from the animal, the instrument to be described here was constructed.

DESCRIPTION OF THE INSTRUMENT

Figure (1) shows a semi-diagrammatic representa-

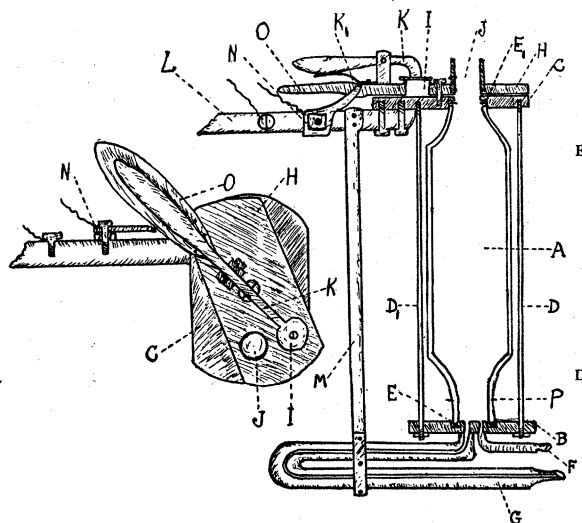


FIG. 1

tion of the instrument; "A" is a glass bulb of approximately 10 c.c. capacity and is clamped between two metal plates "B" and "C" by means of rods "D" and "D₁". Two rubber washers "E" and "E₁" placed between the ends of the glass bulb and the metal plates insure a tight joint. Leading from the lower end of the bulb through the metal plate "B" are two openings, the one leading into an arterial cannula "F" and the other into a glass tube "G" of fine capillary bore and shaped at the free end to facilitate tying into a vein. A hole continuous with that of the upper end of the bulb passes through the upper plate. Above the upper plate "C" is a similar plate "H" held in place by a central screw and capable of being rotated upon plate "C." The movable plate has two openings "I" and "J"; by rotating the movable plate on the stationary one, one or the other of the openings is placed over the hole in the stationary plate leading into the upper end of the glass bulb. One of the openings in the movable plate is connected to a rubber tube through which a definite pressure equal to that in the arterioles of the animal can be applied. The pressure is supplied from the air line and regulated by means of a mercury valve. The other opening in the movable plate is fitted with a release valve "K" with spring "K₁" so that air may be allowed to

escape from the bulb as it is being filled with blood; a small handle "O" is fastened to the moving plate to facilitate rotating it in bringing either opening over the upper end of the glass bulb. A rod "L" to clamp the instrument in place is attached to the upper stationary plate and from this a support "M" passes down to the capillary tube "G." An insulated terminal "N" with a projecting arm is attached to the supporting rod, so that a platinum point on the end of the arm just comes in contact with a similar point on the handle of the rotating plate when the hole connected with the air line is directly over the upper opening of the glass bulb. This insulated terminal and the frame of the instrument is connected in series with a dry cell and a signal magnet. A time clock is made to write on the drum above the signal magnet.

METHOD OF USE

After anesthetizing the animal and injecting sufficient heparin to make the blood incoagulable, the carotid artery and external jugular vein are exposed. The arterial cannula is then inserted into the artery on which a bull dog is placed and the vein prepared for insertion of the capillary tube. After opening the release valve "K" and having the revolving plate in position so that this opening is directly over the upper end of the glass bulb, the bull dog is removed from the artery and the blood allowed to displace the air in the bulb upward until the latter is completely filled with blood, the release valve is then closed. With the blood filling the glass bulb and the capillary tube, the free end of the latter is tied into the external jugular vein. The pressure with which the blood enters the bulb "A" is sufficient to insure its circulation in all parts of the bulb, after which it returns to the external jugular vein by way of the capillary tube "G." A determination may now be taken at any time.

Viscosity determinations are carried out as follows: The bull dog is first replaced on the artery and then the movable plate is quickly rotated by means of the handle "O" until the tube carrying the air pressure is directly over the upper opening in the glass bulb; thus a pressure equal to that in the arterioles is exerted upon the blood and it is forced out of the bulb into the venous side of the circulation. When the blood reaches the mark "P" on the lower neck of the bulb, the rotating plate is quickly moved back to its former position, the release valve "K" is opened and the clamp again removed from the artery. This allows the bulb to again fill with blood and the latter to circulate through the instrument. During the time the blood is being driven out of the bulb by means of air pressure, the arm of the

insulated terminal "N" is in contact with the metal handle of the movable plate; this makes an electrical contact and the signal magnet records the time taken for the blood to be driven through the capillary tube under a known pressure. This time when compared with that required for water under the same pressure gives the relative viscosity of the blood.

It is evident that any number of determinations may be taken without decreasing the amount of blood in the animal. The electrical recording of the time is of advantage in reducing the error due to the reaction time of the experimenter. Because of the short time the blood for determinations remains in the bulb, a bath for temperature control is not thought necessary.

In order to test the accuracy of the instrument, a series of experiments was carried out in which the relative viscosity of 7 per cent. gum arabic was determined by means of this instrument and the same procedure carried out with the Oswald viscosity pipette, water being taken as unity; in thirty determinations with each instrument it was found that the relative viscosity of the gum solution when determined with this instrument was 3.76 while with the Oswald type it was 3.78. These results appear to be well within the range of experimental error. The determinations were made at room temperature and the pressure on the fluid maintained at 70 mm. Hg.

The author is indebted to Professor A. J. Carlson for his helpful suggestions and criticisms of this work and to Mr. F. W. Claassens for his cooperation in construction of the instrument.

RUSSELL A. WAUD

SPECIAL ARTICLES

BALANTIDIA FROM PIGS AND GUINEA-PIGS: THEIR VIABILITY, CYST PRODUCTION AND CULTIVATION

THE following data concerning *Balantidium* occurring in the pig and guinea-pig are deemed of sufficient importance to warrant a report at this time. An abundance of material from the pig has been obtained from two packing plants within several squares of the laboratory, and Dr. W. R. Stokes, of the Baltimore City Health Department, has kindly furnished guinea-pigs for autopsy that died as a result of experimental work. Thus far of the twenty examined, 55 per cent. were infected with *Balantidium*. A colony of rhesus monkeys which also harbor *Balantidium* is maintained by Dr. Carl Hartman, of the Carnegie Institution. This article is a progress report on a problem of host-parasite relations which was suggested to me by Dr. R. W. Hegner.

VIABILITY

According to McDonald¹ (1922) the trophozoites of *Balantidium* of the pig become spherical when the intestinal contents are cooled to room temperature. McDonald also states that they live at room temperature not longer than eight hours. Accordingly, a thermos bottle was used to carry the material to the laboratory from the packing plant. No appreciable rounding was noted when the organisms were examined at room temperature. Therefore, the content of a bottle obtained January 3, 1927, was allowed to cool. Active, apparently normal, trophozoites were found in a sample taken from this bottle the next morning and on every subsequent morning until January 14. The relative numbers did not appear to diminish for about seven days, but then fell off very rapidly. The temperature of the contents of the bottle was taken after fourteen hours, and found to be 20° C. On several other occasions the organisms lived at room temperature for four days and on one occasion for seven days. The viability of trophozoites is also indicated by the fact that water from the trucks in which the pigs were transported from the cars was found to contain them; they remained perfectly normal in appearance at room temperature for twenty-four hours. Feces passed at least two hours previously by ten different pigs were collected from the pens at the packing plant. Trophozoites were found in seven of the ten samples. The pigs had been long in transit from Ohio and the feces were well formed so that they had to be torn apart in water before the trophozoites were freed. The latter appeared perfectly normal and swam about actively.

INFECTIVITY OF TROPHOZOITES

It seems to be the general opinion that ingestion of cysts must occur to set up an infection. (Fantham, Stephens and Theobald,² 1916); but Hegner³ (1926) injected trophozoites from the pig into the stomach of the guinea-pig, and, when the animal was killed one hour later, active, apparently normal trophozoites were found in the stomach, small intestine and cecum. This experiment has been repeated with success.

¹ McDonald, J. D. 1922. "On *Balantidium coli* (Malmsten) and *Balantidium suis* (sp. nov.) with an Account of their Neuromotor Apparatus. Univ. Calif. Pub. Zool., 20: 243-300.

² Fantham, H. B., Stephens, M. D., and Theobald, M. A. 1916. "The Animal Parasites of Man," 900 pp. New York.

³ Hegner, R. W. 1927. "Host-Parasite Relations between Man and His Intestinal Protozoa." The Century Co., New York. (In press.)