**XLX. THE EFFECT OF AIR, WHICH HAS BEEN
EXPOSED TO THE RADIATIONS OF THE
MERCURY-VAPOUR QUARTZ LAMP, IN
PROMOTING THE GROWTH OF RATS,
FED ON A DIET DEFICIENT IN
FAT-SOLUBLE VITAMINS.**

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Introductory.

It has already been demonstrated [Hume, 1922; see also Goldblatt and Soames, 1922] that young rats fed on a diet deficient in fat-soluble vitamins, grow normally for a much longer period, when they are irradiated with the mercury-vapour quartz lamp, than when not so treated. Experiments by Kestner [1921, 1922], to which attention was directed by Professor Hans Meyer, Vienna, suggested the possibility that exposure to a source of ultra-violet light might not be the only means of thus inducing growth, but that exposure to air through which such light had passed might also be effective. Kestner sought to disentangle the factors which give to a mountain climate its well-known health promoting properties. It had already been observed [Laquer, 1913, 1919; Weber, 1919] that in dogs rendered anaemic, the red blood corpuscles are regenerated more quickly at an altitude (Monte Rosa) than in the plains, an effect generally attributed to the reduced oxygen pressure at high altitudes. Kestner made his tests with dogs rendered anaemic by bleeding or by injection of pyrocin, and he observed the rate of regeneration of red cells in control animals and in those under the influence of (a) reduced atmospheric pressure, (b) the light from a naked carbon arc lamp, and (c) the air drawn from the neighbourhood of such a lamp. Reduced pressure produced no more rapid regeneration than occurred in control animals, but the direct rays of the lamp or air drawn from the neighbourhood of the lighted lamp both caused the regeneration to be more rapid and complete. It seemed therefore worth while to test whether air which had been

exposed to a source of ultra-violet light, also possessed the power of prolonging normal growth in rats fed on a diet deficient in fat-soluble vitamins. This was tested by means of the following experiments. Experiment 1 was performed in Vienna and experiments 2-4 at the Lister Institute.

The diet deficient in fat-soluble vitamins had the following composition: heated caseinogen, 180 g.; maize starch, 520 g.; hardened cotton-seed oil, 150 g.; salt mixture, 50 g.; marmite (yeast extract), 60 g.; lemon-juice, 50 g.; water, 600 g. The salt mixture had the following composition: sodium chloride, 51.9 g.; mag. sulph., 164.0 g.; sodium acid phosph., 104.1 g.; potassium phosph. 286.2 g.; calcium phosph., 162.0 g.; calc. lact., 390.0 g.; ferric citrate, 35.4 g.

All rats, both treated and controls, were placed upon the deficient diet at a weight of about 50 g., and treatment, where such was instituted, began from that time or a few days later. Wherever possible controls were taken from the same litter.

The mercury-vapour quartz lamp used for the Vienna experiments was of the Hanau pattern; it was always allowed to run for at least five minutes before being used. No estimate was made of its deterioration since purchase, but this was probably great. The lamp used for the Lister Institute experiments was a 74 pistolette quartz lamp made by the Hewittic Electric Company, using a current of 3.5 ampères and emitting rays whose shortest wave length was 230μ .

The treated rats and their controls were kept, each animal separately, in 8-litre glass cylinders, with sawdust at the bottom. The treatment with irradiated air was carried out as follows. Every second day, the rats were removed from the glass jars and left in the animal room, while the jars were conveyed to the room containing the mercury-vapour lamp, which in the Kinder Klinik was at the extreme other end of the building, and, in the Lister Institute, on another floor. The jars were placed below the lamp, with their bases about 80 centimetres distant from it, so that the light shone straight down into them. They remained in this position for ten minutes, were then covered with glass lids and conveyed back to the animal room. The animals were put in and the covers replaced to hinder diffusion of the air. *After ten minutes* the covers were removed and not replaced but the animals continued to live in the jars until the next treatment.

Experiment 1. Three animals, all from the same litter of three, were treated with irradiated air. One had been for seven days and the other two for four days, on the deficient diet, before treatment began. The weight curves, however had not begun to flatten off and even if they had done so, previous experiment [Hume, 1922] has shown that for a considerable time after being placed on the deficient diet, rats are capable of responding to the stimulus of radiation and resuming normal growth.

Control animals from the same litter were not available but other rats bred in the same way and placed upon the deficient diet a little previously,

Chart 2. Experiment 2. Weight curves of rats fed on a diet deficient in fat-soluble vitamins.
A. Treated with air, exposed to the mercury-vapour quartz lamp in glass jars for ten minutes every second day.
B. Untreated controls.
From X, the animals exposed directly to the rays of the lamp, for ten minutes daily.
(Members of the same litter are designated by the same small letter.)

as good as that of the directly radiated animals and is much better than that of the untreated controls. In the controls normal growth continued for a week, in the rats treated with irradiated air it continued for four weeks, and in the animals directly irradiated for from 3-7 weeks.

Experiment 2, Chart 2. This experiment was similar to experiment 1. Two rats from one litter, and four rats from another litter were divided into two groups of three, so that the two litters were equally represented in each group. One group of three, Chart 2 (A), was treated with irradiated air, and the other group of three served as controls, Chart 2 (B). The same superiority of the treated rats (A) over the untreated controls (B) is apparent, as in experiment 1. The controls in this case are slightly more strongly growing and the growth of the strongest control is as good as that of the weakest treated rat. Reference to the previous papers of Hume and of Goldblatt and Soames, shows, however, that this may also occur when rats are irradiated directly.

Chart 3. *Experiment 3.* Weight curves of rats fed on a diet deficient in fat-soluble vitamins.

- A. Treated for one hour daily, with air drawn from the neighbourhood of the mercury-vapour quartz lamp. From X, treatment changed, animals treated with irradiated air in jars.
- B. Untreated controls.

(Members of the same litter are designated by the same small letter.)

On the 60th day of experiment, when growth had practically ceased in every case all the six rats were exposed directly to the rays of the lamp for ten minutes every day. All made a small response and resumed growth for a period, which, except in the case of one control, was only of very short duration. The response of the controls was however the greater.

A dose of freshly irradiated air, given every second day, did not therefore evoke quite the maximum growth response, capable of being produced by direct irradiation of the animal, but it did produce a very marked prolongation of normal growth, beyond that shown by controls. A dose of irradiated air, given more often than once every second day, might be able to produce the maximum response.

Experiment 3, Chart 3. In this case the rats were exposed to air, drawn from the neighbourhood of the lighted mercury-vapour lamp, the animals being shielded from the illumination from the lamp.

A 4.5 ampère Hanau lamp was used. The lamp was surrounded by an iron shield. This iron shield which was earthed by a wire to the gas pipe was fairly air-tight, except for the ventilation holes above and below and the opening in front for the illumination to pass. The upper ventilation holes were closed with corks and a tinned funnel (10 cm. diameter) was soldered over the illumination aperture. To the stem of the funnel, 11 feet (3.3 metres) of glass tubing of 0.8 cm. outer bore, was attached, the other end of which passed through a light-tight box. The lamp was kept cool by a wet bandage around the shield.

The rats were placed singly in large bottles enclosed in a light-proof box. The bottles were closed with rubber bungs, admitting inlet and outlet tubes. Air was sucked through the whole system by a water pump. The ventilation was at the rate of 20 litres per hour. Ozone was produced in the neighbourhood of the lamp and could be detected in the bottles and at the outlet; the amount diminished as the distance from the lamp increased. After the lamp had been lit for 15 minutes, the box containing bottles and rats was brought into the room, joined up and left there for one hour daily.

Six rats were used for the experiment, all belonging to the same litter. Three were exposed to the air from the lamp and three were left untreated and used as controls.

Chart 3 shows the weight curves of the three treated rats (*A*) and of the three controls (*B*). After 20 days it was apparent that normal growth would not be prolonged by treatment with irradiated air in this way; the growth of the treated rats was inferior even to that of the controls, which in this instance showed remarkably strong growth. On the 20th day treatment with air drawn from the lamp was stopped, and treatment in jars of radiated air, as described under experiment 1, was instituted. The jars were freshly irradiated every day for ten minutes. This treatment was continued for 20 days and a moderate growth response was obtained (see Chart 3 (*A*), curves from *X* onwards).

It was therefore concluded that air drawn from the neighbourhood of the lamp, by means of such an apparatus as that described, does not possess the power of prolonging normal growth in rats, fed on a diet deficient in fat-soluble vitamins. The inferiority in the growth of the rats treated, to that of the controls, suggests that some factor, actually detrimental, was at work; this may have been the ozone. At any rate it seems safe to conclude that the ozone is not the growth stimulating factor.

Experiment 4, Chart 4. This experiment was a repetition of Nos. 1 and 2. In addition an extra series of jars was irradiated in the same way, but the air was blown out and fresh, not irradiated, air was admitted before the rats were placed in the jars. The jars were blown out with 20 strokes of a bellows, a process occupying a very short time, and only delaying the entrance of the rats into the jars by about 40 seconds. All jars were irradiated every day, instead of every second day as in experiment 1. Four litters of rats were used

Chart 4. Experiment 4. Weight curves of rats fed on a diet deficient in fat-soluble vitamins.

A. Treated in irradiated jars containing irradiated air.

B. Treated in irradiated jars containing air not irradiated.

C. Untreated controls.

(Members of the same litter are designated by the same small letter.)

and each litter was divided equally between the three experimental groups, *i.e.* (*A*) treatment in irradiated jars, containing irradiated air, (*B*) treatment in irradiated jars containing air, not irradiated, and (*C*) control animals undergoing no treatment. Each group contained five animals. Chart 4 shows the weight curves of the three groups. The result is quite unequivocal; the rats treated with irradiated air (*A*) show prolongation of normal growth, while those occupying irradiated jars from which the irradiated air has been displaced, (*B*) behave like the untreated controls (*C*).

It is therefore evident that the growth promoting property lies in the irradiated air itself, and not in any property which might have been acquired by the glass.

SUMMARY.

(1) Rats fed on a diet deficient in fat-soluble vitamins, when kept in glass jars which had been exposed to the mercury-vapour quartz lamp for ten minutes every second day, showed prolongation of normal growth over controls not so treated.

(2) Rats exposed to the air containing ozone drawn over the quartz lamps and passed through 3 metres of glass tubing, 0.8 cm. bore, showed poorer growth than the control animals. The same rats, subsequently treated in glass jars of irradiated air gave some growth response.

(3) Rats placed in irradiated jars from which the irradiated air had been displaced, showed no prolongation of normal growth.

These results are analogous to the accelerated regeneration of red blood corpuscles in anaemic dogs observed by Kestner. In both cases the effect was obtained by exposure of the animals to air through which ultra-violet light has passed, as well as by exposure to the light itself. Kestner used a 40 ampère naked carbon arc lamp and the air was drawn from the neighbourhood of the lamp and passed through a box in an adjoining room in which the dogs were placed. The animals were supplied with this air for 3 to 5 hours daily.

Kestner concludes that as a result of sun radiation or radiation from the carbon arc lamp, substances are formed which, if breathed, stimulate the formation of blood corpuscles. He further suggests the possibility that compounds of nitric oxide, formed when the lamp is alight, are responsible for the action.

In the present experiments, a mercury-vapour lamp enclosed in quartz was used. This is not believed to produce nitric oxide. In experiment 3 the air drawn away from the lamp was found to possess no growth-promoting activity, but it may have been exposed for too short a time to the lamp or the character of the apparatus used may have been such as to deprive it of this activity before it could reach the rats. The negative result obtained in experiment 4 when the irradiated air in the jars was displaced makes it plain

that it is *the air and not some property impressed on the glass jars which is active*.

Ozone was present in the air in experiment 3 which gave a negative result; it is therefore clear that ozone is not responsible.

The other effect produced on air by ultra-violet radiation is a small degree of ionisation, a change which disappears rapidly. Air ionised in capacious jars, by reason of the large volume and less relative surface exposed to glass, would remain ionised somewhat longer than when drawn through glass tubes, with rubber junctions. This may explain the positive result obtained with the former method and the negative one obtained with the latter.

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