found decrease in the number of mitoses in marrow cells 2 hours after irradiation. The number of mitoses in the cultures irradiated with the doses of 250-2500 r units was reduced to approximately 20% of that in non-irradiated controls. In cultures irradiated with 5000 r units the mitoses disappeared almost completely at this time.

There was a definite recovery of the mitotic activity 4 hours after irradiation in cultures treated with 250 and 500 r units. The process of the recovery in cultures irradiated with these doses continued and the mitoses reached 8 hours after irradiation values equal to those of the controls. Cultures irradiated with 1000 r units showed no significant recovery after 4 hours, but only after 8 hours; in this case also the recovery was complete. After 24 hours the culture irradiated with 1000 r units and less showed an abundance of mitoses and normal cellularity.

A distinctly different behavior was observed in cultures irradiated with 2500 and 5000 r units. In cultures treated with these doses the recovery took place 8 hours after irradiation; it was incomplete, the number of mitoses rising only to 30% of the values in controls. No further increase of the mitotic count could be observed 24 hours after irradiation. In contrast to the cultures treated with doses of 1000 r units and less, the explanted bone-marrow fragments irradiated with 2500 and 5000 r units, presented after 24 hours a picture of complete or almost complete aplasia.

The mitotic behavior of irradiated bone-marrow cultures in relation to the control cultures is represented in Fig. 1.

Summary. The effect of direct irradiation with X-rays on mitoses in cultures of rabbit's bone-marrow was investigated. The X-rays in all doses applied (250, 500, 1000, 2500, 5000 r units) produced at first a profound decrease in the number of mitoses. In cultures irradiated with doses of 1000 r units and less the decrease in mitoses was followed by complete recovery of the mitotic activity. The recovery of mitoses in cultures irradiated with 2500 r units and more was only incomplete and the irradiation resulted in aplasia of the bone marrow.

15005

Effect of Drop Size and Wall Solution Concentration on Determinations Made with the Hill-Baldes Method.

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The Hill-Baldes vapor tension method of determining osmotic pressure has been used extensively for determining the osmotic activity of biological fluids. This method involves a comparison of the rates of evaporation from or condensation on drops of the sample and of a reference solution when placed on opposite junctions of a thermocouple enclosed in a chamber whose walls are lined with moistened filter paper. The deflection of the galvanometer to which the thermocouple is connected is a measure of the difference in vapor pressure and therefore of osmotic pressure of the two solutions, and is calibrated by using 2 known or reference solutions.[1]

During a recent investigation of the osmotic activity of gastrointestinal fluids following the ingestion of distilled water,[2] osmotic activity readings were made at intervals over a period of from one to 3 days following drop deposition on the thermocouple loops, whereas ordinarily readings are completed within one

[1] Roepke, R. R., and Baldes, E. J., *J. Cell. and Comp. Physiol.*, 1942, **20**, 71.

[2] Follansbee, R., *Am. J. Physiol.*, in press.

hour of drop deposition. In the course of these experiments two unexpected difficulties arose: (1) In some cases the apparent osmotic activity of salt solutions fell to values below those of the reference solutions with which they were being compared, and (2) duplicate samples failed to give consistently identical osmotic activity readings throughout the course of a single experiment.

It appeared probable that factors, control of which may be safely neglected over shorter time intervals, were operating to produce these anomalous results in the case of the longer time intervals. From a consideration of the theory of the method,[3] it is seen that (1) the size of the drops and (2) the difference in osmotic concentration between the drop solution and that on the walls of the thermocouple chamber are such factors. The drops on the thermocouple junctions and the solution lining the chamber wall are continuously approaching diffusion equilibrium with one another by exchange of water. Since the volumes and surfaces of the drops are negligible compared with those of the wall solution each drop may be considered independent of the other and the water exchange to be dependent solely upon the rate at which the drops approach the concentration of the wall solution.

When the wall solution is lower in osmotic activity than the drop the latter will be progressively diluted with time and *vice versa* with the opposite condition. Other conditions being constant, drop size and concentration difference between drop and wall solution determine the rate of equilibration, the smaller the drop the greater the per cent rate of equilibration, and the larger the concentration difference the greater the change in osmotic activity for a given degree of approach toward equilibrium. If the reference and sample drops are of unequal size, they will become diluted at different rates. Since the osmotic activity reading at any given time is a measure of the difference between the two drops at that time, such readings will not represent the osmotic activity differences between the drops at the time of drop deposition, if unequal rates of dilution occurred.

It seemed therefore advisable, in an attempt to explain the difficulties mentioned above, to perform experiments to test the effect on the osmotic activity readings of the concentration of the solution on the walls of the thermocouple chamber and of the size of the drops. The present paper reports the results of these experiments.

Procedures and Results. The apparatus and technic employed were essentially the same as those described by Baldes and Johnson.[4] Four pairs of thermocouples are available in a single chamber and simultaneous duplicate determinations were made in each experiment. Two micrometer burettes[5] with needles attached were set up vertically so that drops of known volume could be placed on the thermocouple loops. Drops containing 0.0012 cc were designated as "small" drops and those containing 0.0032 cc as "large" drops. The experiments were done with known concentrations of sodium chloride in water.

Fig. 1 presents a comparison of duplicate determinations of the rates of equilibration of small and large drops. In this case the sample solution (240 mM NaCl) was the only solution changing concentration with time since both the reference and wall solutions were the same (220 mM NaCl). It will be noted that in accordance with expectations the "large" drops (Fig. 1, Curve I) equilibrated more slowly (about 25% equilibration reached in 30 hours), than did the "small" drop (Fig. 1, Curve II) which came to complete equilibrium in 30 hours or less. It is thus apparent that unless drop size is controlled, large discrepancies between duplicate determinations will occur.

Fig. 2 shows a similar experiment except that the wall solution was 160 mM NaCl. In this case the results are also in accordance with expectations. Since the wall solution was more dilute than either reference or sample drops, both of the latter are approaching the wall solution in concentration, but at different rates due to different drop size. When the sample (240 mM NaCl) drop was

[3] Baldes, E. J., *Biodynamica*, 1939, **46**, 1.

[4] Baldes, E. J., and Johnson, A. F., *Biodynamica*, 1939, **47**, 1.

[5] Dean, R. B., and Fetcher, E. S., Jr., *Science*, 1942, **96**, 237.

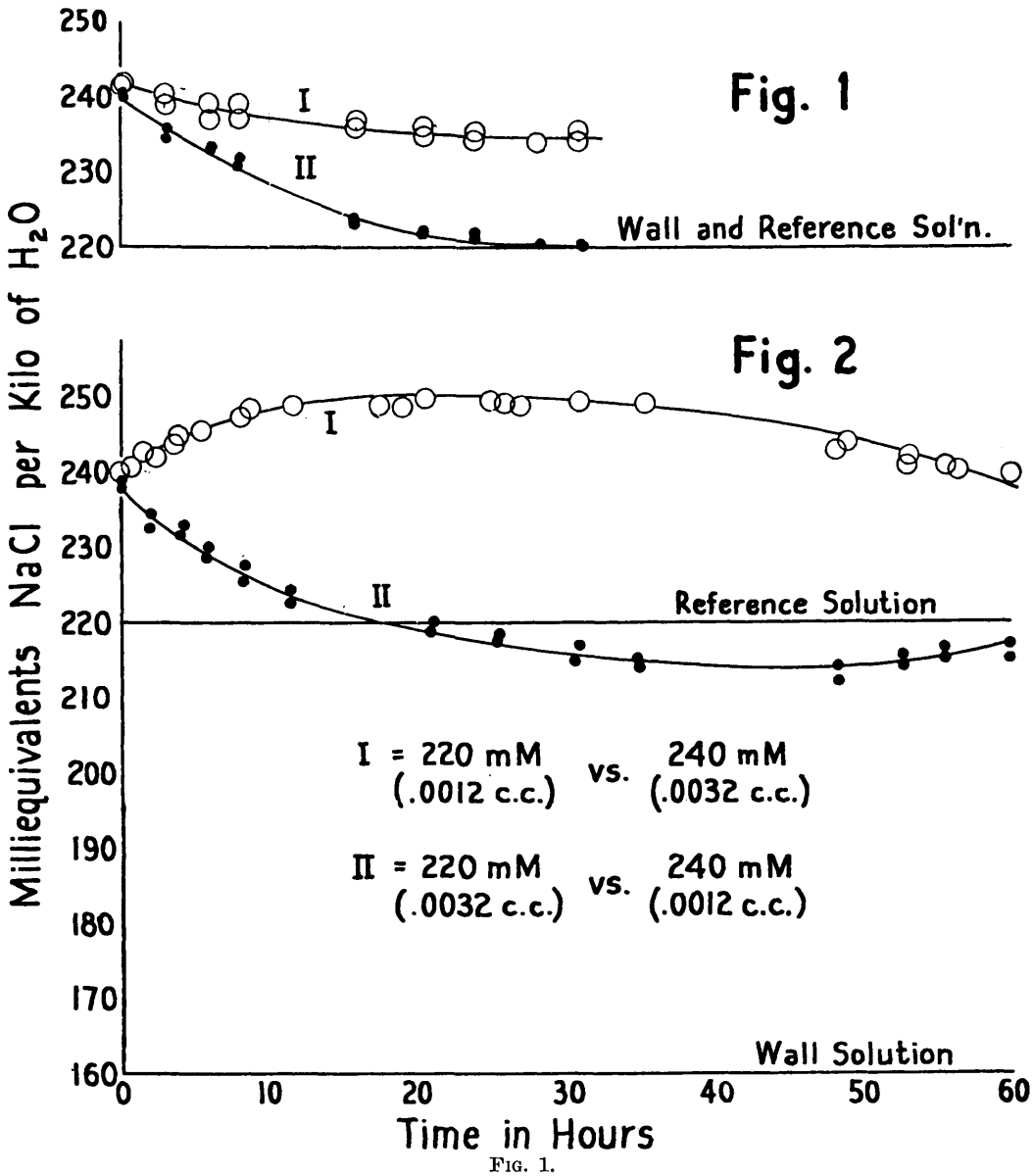

FIG. 1.

Influence of drop size on the osmotic pressure readings when the wall and reference solutions are identical.

FIG. 2.

Artificial osmotic activity readings observed when the unknown and reference drops are of unequal size and the wall solution is more dilute than the reference solution.

large compared to the reference drop (220 mM NaCl, Fig. 2, Curve I) the sample was diluted more slowly than the reference drop so that the concentration difference between the 2 increased for a time. This was read as an increase in osmotic activity of the sample drop. However, when the reference drop was the "large" one, it was diluted more slowly than the sample drop, so that the difference in concentration decreased quite rapidly. This was read as a decrease in osmotic activity (Fig. 2, Curve II). The "small" drop, in this case 240 mM NaCl, was diluted so much faster than the "large" drop that after eighteen

hours the 240 mM drop had become more dilute than the 220 mM drop. This was read as a fall below the reference solution.

It is noted that in both experiments described above (Fig. 1 and 2) the initial readings gave correct osmotic pressure values. This is in agreement with Roepke's findings[6] on the effect of drop size on osmotic pressure determinations made with the Hill-Baldes vapor tension apparatus. He states that "it is not necessary under ordinary conditions with dilute solutions that the drops be of exactly the same size." The experiments described in the present report indicate that precautions regarding equal drop size and identical reference and wall solutions must be taken if osmotic pressure readings are to be made over long periods of time after drop deposition on the thermocouple loops. Under some conditions it may be advantageous to control these factors even when the time intervals involved are short.[7] It is interesting to note that the influence of these variables is exhibited in an exaggerated way when the drops consist of a mixture of deuterium oxide and water.[8]

Summary. Experiments are described that test the effect on osmotic pressure readings made with the Hill-Baldes vapor tension method, of the size of the drops placed on the thermocouple loops and of the concentration of the solution placed on the walls of the thermocouple chamber. Evidence is presented indicating that when readings are made over long periods of time after drop deposition on the thermocouple loops the drops must be of equal size and the reference and wall solutions must be identical if accurate osmotic pressure values are to be obtained throughout the course of the experiment.

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15006 P

The Cellular Transfer of Cutaneous Hypersensitivity to Tuberculin.

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In studies on experimental drug allergy, it has been found that specific hypersensitiveness of the "delayed type" is transferable to normal guinea pigs by means of cells in exudates recovered from sensitized guinea pigs.[1] Resemblances between the delayed type of reaction to drugs and the classical tuberculin reaction have prompted investigation as to whether cells from tuberculin-sensitive animals may likewise transfer tuberculin sensitivity. The experiments show that guinea pigs receiving such cells acquire for a limited time a skin hypersensitivity that exhibits the essential features of the typical tuberculin reaction.

Guinea pigs were rendered hypersensitive to tuberculin by subcutaneous injection of killed human tubercle bacilli suspended in paraffin oil,[2,3] usually mixed with vaseline; each animal received 0.5 to 2.5 mg of dried tubercle bacilli in a total inoculum of 1 cc. Between 5 and 9 weeks later, the cutaneous

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3 Freund, J., Casals-Ariet, J., and Hosmer, E. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 509; Freund, J., Casals-Ariet, J., and Genghof, D. S., *J. Immunol.*, 1940, **38**, 67; Freund, J., and Gottschall, R. Y., *Arch. Path.*, 1942, **34**, 73.