taneously into the right lower quadrant of the abdominal wall of each animal.

During the 5 months since the infected material was inoculated the following observations were made: Mycobacteria exposed to 17°C. and 37°C. produced palpable lesions at the injection-site in all animals within 3 months; organisms heated to 39°C. caused lepromata in 7 of 8 surviving rats in 3 months; 60% of the animals given material kept at 41°C. had lesions at the end of 3 months and 75% of the survivors within 4 months. Six of 7 surviving rats injected with the leprous suspension exposed to 43° had palpable leprous masses at the end of 4 months. Temperatures of 50° and 60°C. for 5 hours are sufficient, apparently, to attenuate the *Mycobacteria* and prevent the appearance of lesions, since no animal receiving this material showed signs of leprosy during the observation period of 5 months.

Weight gains were noted only in groups not developing lepromata, *i. e.,* 50°C. (average gain, 27 gm.) and 60°C. (average gain 180 gm.). In the controls given material kept at room temperature, however, an increase due to growth was noted. Intercurrent infection, usually pneumonia, accounted for the death of 26 of the 70 rats originally injected.

Summary. Temperatures in excess of 43°C. for 5 hours are required to kill *Mycobacterium leprae muris in vitro,* using rats as a test animal to determine viability over an observation period of 5 months. Fifty degrees C. for 5 hours seems sufficient to attenuate this *Mycobacterium.*

8308 P

Hemolytic Complement Albumin-Globulin Ratio.

M. C. TERRY. (Introduced by W. H. Manwaring.)

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If fresh, cell-free, guinea pig serum in a test tube is repeatedly frozen and thawed without shaking or inverting the tube there will be seen, after a few freezings and thawings, a difference between the upper and lower portions of the tube. If the serum is tinged with hemoglobin the lower portion will be deep red and the upper portion colorless. If there is only a trace of hemoglobin an indica-

tion of what takes place may still be shown by gently tilting the tube back and forth whereupon a transverse movement of heavy oily-looking streaks is seen, somewhere below the middle of the tube, between an amber colored lower portion and a colorless upper portion. If the lower portion is recovered and titered for complement content against sensitized sheep cells this portion will be found to have a higher titer than that of the original whole serum, the difference being greatest if only the extreme lower portion of the concentrated serum be employed.[1]

In one such experiment the contents of the tube, immediately after the last thawing, was divided into 2 equal upper and lower portions and nitrogen determinations were done on the 2 halves and on the original whole serum in addition to complement titrations. The complement titer was higher and the total nitrogen content was greater in the lower half than in the whole serum. The difference in complement value was as 100 to 133; the difference in albumin as 100 to 230; in globulin as 100 to 150. The gain in complement, therefore, was associated with a change in the albumin:globulin ratio, the change consisting in a relative increase in albumin and a relative decrease in globulin. Since the experiment involved no procedure which would seem likely to have any denaturing effect these findings seem significant. The method of freezing and thawing has been employed for concentration of non-colloid solutions and in this experiment with complement the effect of what approximates distilled water in the upper half of the tube must be considered. It may be noted that the effect is the opposite of that produced by the dilution of serum with distilled water, a procedure which is destructive of complement function.[2]

Following the experiments described the investigation was continued by dialysis of guinea pig serum. The globulin precipitate was taken up to volume in normal salt solution and the supernatant albumin fraction was brought to isotonicity by added salt and heated at 55° for 15 minutes. Additions of these fractions were made to fresh serum which was then titered as complement. It was found that albumin addition raised the titer slightly while globulin addition lowered it considerably and in larger quantity extinguished it completely. Whether albumin and globulin from other sources would similarly affect guinea pig complement is a matter now under investigation.

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1 Plant, Arthur S., *Br. Med. J.*, 1933, **2**, 414.

2 Sachs u. Teruuchi, *Berl. kl. Woch.*, 1907, 16, 17, 19.

Guinea pig serum was now kept before an electric fan until the volume was reduced to about 1/10 by "pervaporation".[3] The gross change was obviously the removal of water. The complement titer of the serum thus highly concentrated was only about doubled.

Since pervaporation presumably made no change in the relation of the various protein fractions to each other and resulted in a relatively slight increase in complement titer while freezing and thawing caused a demonstrable change in those relations and was accompanied by a notable increase in titer it would appear that the increase in titer in the latter case was due to the changed relation of protein fractions, the demonstrated change being a relative increase of albumin and a relative decrease of globulin.

8309 P

Vacuolization During the Water Exchanges of Cells.

JAMES L. LEITCH. (Introduced by S. C. Brooks.)

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In a study of the water exchanges of the eggs of the sea urchin, *Echinometra lucunter,* the appearance of vacuoles was noted during one phase of the swelling process. When single eggs of this species were observed while swelling in 60% sea water, 2 different equilibria were found during each of which the measurements of the egg diameters remained constant for a period of from 20 to 30 minutes. The first of these occurred after approximately 60 minutes' exposure to the experimental solution and the second after 120 minutes, the eggs now exhibiting a somewhat smaller volume. The shrinkage occurring between these 2 equilibria was accompanied by active vacuolization, the vacuoles appearing in the central portion of the egg and migrating to the cortical layer. Although the emptying of these vacuoles to the outside was not seen, it was inferred from the fact that no accumulation of vacuoles could be detected at the periphery even though additional vacuoles were continually migrating in that direction.

Just[1] raised the question whether the consideration of an egg as

[3] Farber, Lionel, *Science,* 1935, **82**, 158.

[1] Just, E. E., *Protoplasma,* 1930, **10**, 24.