and those which either had not developed or had just started to develop. When no ova greater than 1 cm in diameter were observed the blood calcium level was between 13.0 mg and 7.5 mg. Eight of the group of 30 pullets had been deprived of the antirachitic factor for a period of 8 months before killing and consequently were not actively producing eggs, although they had not started to molt. In spite of this deprivation, the presence of large ova was accompanied by a blood calcium level greater than 13.0 mg.

Ten pullets were maintained on the same basal ration for a period of four months longer; these were of the large group of which 30 had been killed and they had received their antirachitic factor throughout the eight-months laying period in the form of irradiated ergosterol, in an amount biologically equivalent to 10 times the 2 per cent. of cod-liver oil which is frequently used in poultry rations. During July and August the amount of irradiated ergosterol was doubled, but this did not stop the waning egg production associated with molting. Early in September, the feeding of 2 per cent. of cod-liver oil was begun in place of the ergosterol and continued until the end of the experiment. Observations of the ova and blood calcium of the 10 individuals were made early in the month of October when the birds were in a molting condition. Nine had no ova larger than 1 cm in diameter, and their blood calcium was 13.7 mg or below. One individual showed a blood calcium of 16.0 mg and 4 large ova were observed.

On November 5, 1929, five hens in a molting condition, but which had been exposed to all the sunlight available each day for a period of 6 weeks, were killed for observation. No ova greater than 1 cm in diameter were noted, and the blood calcium level was below 13.0 mg except in one case in which a value of 13.5 mg was obtained. Although an adequate amount of the antirachitic factor had been supplied to these individuals a high blood calcium did not prevail and ova greater than 1 cm in diameter were not present. In an earlier preliminary experiment (unpublished data) it was found that the feeding of daily doses of irradiated ergosterol to hens not in a molt, but which were not in active egg production because of a deprivation of the antirachitic factor, caused active production to take place.

The data show that the presence of large ova and high blood calcium does not always indicate active egg production, and suggest that the antirachitic factor may not be necessary in the development of ova, at least, it may not be the essential factor involved in the development, and that the antirachitic factor may not be the sole factor in causing high blood calcium.

Although a biological test, the use of the white rat, indicated that the basal ration was devoid of the antirachitic factor, it is realized that traces of this factor may have been present in the ration which permitted only a slow development of ova and occasional production of an egg.

Riddle and Reinhart[1] have shown that high blood calcium prevails in female pigeons at each ovulation period, and Hughes, Titus and Smits[2] report that high calcium prevails in hens which are in production, and low calcium in those which are molting. Our results confirm those of the above investigators with the exception that high blood calcium is not always associated with active egg production. Recently, Buckner, Martin and Hull[3] reported high calcium values for actively producing hens and for those not in active production, whereas molting hens showed a low value. Our records also confirm this report.

Whether the development of ova caused the blood calcium to rise or whether an increase in blood calcium stimulated ova formation will have to be investigated further, but it should be noted that high blood calcium was never found unless developed or developing ova were present.

It is of interest to note that Hess, Bills, Weinstock and Rivkin[4] found the blood calcium of the cod to be high at the spawning season although the eggs are without shells. In the case of the hen the presence of ova, although eggs with shells were not being produced frequently, was accompanied by a high calcium level.

Observations of 49 hens and pullets in June of this year have confirmed our previous results, and an extended report of this investigation is being prepared for publication.

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THE QUANTITATIVE DETERMINATION OF BACTERIOPHAGE

IN a recent paper[1] the writer described a comparative method for the quantitative determination of bac-

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[3] G. D. Buckner, J. H. Martin and F. E. Hull, *Am. J. Physiol.*, 93: 86, 1930.

[4] A. F. Hess, C. E. Bills, M. Weinstock and H. Rivkin, *Proc. Soc. Exp. Biol. and Med.*, 25: 349, 1928.

[1] A. P. Krueger, "A Method for the Quantitative Determination of Bacteriophage," *Jour. Gen. Physiol.*, 1930, 13: 557–564.

teriophage. Briefly, the method depends upon the fact that with a set concentration of growing phage-susceptible bacteria and varying concentrations of phage, the time of lysis is a function of the initial phage concentration. An arbitrary turbidity standard is chosen as an end-point and the periods of time required to reduce to this level the dense bacterial suspensions in unknowns and in dilutions of "Standard phage" are recorded. (Standard phage is readily prepared in quantity. Its titer is defined in terms of arbitrary activity units and it may be kept at 4° C. for months without change in titer). By plotting the time required for the unknown to reduce the suspension to the standard end-point, the activity of the unknown solution may be calculated in terms of the activity of the standard phage with an accuracy of about ± 3 per cent.

Necessary conditions for satisfactory results are: (1) Constant temperature; (2) mechanical rocking of the test series to avoid settling of the bacteria; (3) accurate determinations of numbers of bacteria both in setting up the test and in reading bacterial concentrations during lysis;[2] (4) careful dilution technique and accurate time measurements.

Routine daily use of the method has brought out the following points in its favor: (a) Twenty to thirty unknowns may be conveniently run at once; (b) time required for the entire test is < 5 hours; (c) results are accurate to within ± 3 per cent., a figure based upon an analysis of the last 60 series run in this laboratory; (d) the procedure is definitely more reliable and is more easily carried out than either the plaque count or dilution technique ordinarily used in determining phage titers; (e) kinetic analysis of the phage-bacterium reaction predicts the relationship between phage concentration and time of lysis on which the quantitative determination depends.[3]

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THE MECHANISM OF ENHANCEMENT OF INFECTIONS BY TESTICLE EXTRACT[1]

IN a series of publications Reynals has reported the observation that testicle extract to a marked degree, and certain other organs to a less extent, enhance the lesions produced by vaccine virus and staphylococcus infections.[2] Hoffman has obtained similar results with several other viruses[3] and Pijoan with a number of other bacteria.[4] A possible explanation of the enhancement mechanism was suggested by the observation that the wheals resulting from intracutaneous injections of the infectious agents with testicle extract disappeared more rapidly than those resulting from injections of the agents with inactive organ extracts. On the basis of this clue a large series of experiments has been carried out in order to determine the effect of testicle extract on the diffusion of inert substances in the skin.

For the main experiments India ink was used as the test substance. A mixture of this suspension was made with equal amounts of the various organ or tissue extracts, and 0.25 cc of each mixture was injected intracutaneously in the shaved skin of rabbits. The maximum spread of all mixtures was reached within an hour, so this period was selected for measurements. The results of a number of experiments were as follows. The average size of the area of spread for India ink-testicle extract mixture was 4.5 x 3.5 cms, while that for the control of India ink and Ringer's solution was 2.5 x 2.1 cms. The other extracts with India ink gave less striking differences. Kidney and to a less degree spleen extracts gave spreads larger than the controls, but rat and rabbit serum seemed to be without effect on the diffusion of the ink.

Another point noted, which may have a bearing on the enhancing power of testicle extract, is that the ink particles were not only spread through a wider area under the influence of the factor, but great numbers of the particles were found either in the cells or adhering to the cells. With the inert extracts the injected particles lay in the tissue spaces with no especial contact with the cells. This suggests a second effect of the enhancing substance, namely, an increased permeability of the local host cells. The activity of the testicle extract in enhancing infections as well as increasing the spread of inert particles is destroyed by heating at 60° for 30 minutes.

The tentative conclusion indicated by these observations is that the enhancing property of testicle extract on infections is at least partly due to the fact that it increases the area of spread of the injected material and increases cell permeability. The details of the experiments with a fuller discussion will be given in a subsequent publication.

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