2.4 gm. of solid material, since the bacteria contain about 90% water. Mussels have been maintained in this laboratory for 16 months on an exclusive diet of bacteria. Professor T. Kincaid[6] has maintained oysters for several months with nothing to eat except bacteria. The oysters appeared to develop normally and their glycogen-content increased.

To date a total of 30 different new and undescribed species of marine bacteria[5] have been filtered from prepared sea-water suspensions by mussels, ingested and probably digested. In general, bacteria washed free of their metabolites do not seem to injure mussels unless so many are added that their respiration vitiates the water. In the presence of appreciable concentrations of bacterial nutrients such as peptone, the products of bacterial metabolism are usually toxic for the mussels.[1] The peptone itself is not toxic as indicated by the fact that mussels survive for several days in peptone solutions at refrigerative temperatures which retard bacterial activity.*

Most of the bacteria fed to mussels were alive. Heat-killed bacteria were also ingested by the mussels but they were not tolerated in as large doses as the living ones. This is attributed to the vitiation of the water by other bacteria which are always associated with the mussels and which multiply at the expense of the organic matter from the heat-killed bacteria. Bacteria known to be pathogenic for animals have not been fed to the mussels.

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Augmentation of the Gonadotropic Hormone from the Pregnant Mare.*

ALLEN LEIN. (Introduced by Bennet M. Allen.)

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Several investigators have described the so-called augmentation phenomenon, which has recently aroused considerable interest in its applications to the study of the gonadotropic hormone obtained from the blood and certain other tissues of the pregnant mare. Cole and

[6] Personal communication, University of Washington, Seattle.

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Hart[1] have shown that pregnant mare serum, when combined with pituitary synergist, is able to augment the action of the latter substance. However, non-pregnant serum, having no gonadotropic activity when tested on immature rats, was also found to augment the pituitary synergist, which is known to have follicle-stimulating activity. It was thus believed that both pregnant and non-pregnant mare serum perhaps contain a luteinizing hormone which is effective only when combined with pituitary synergist. However, later work[2] confirmed the findings of Maxwell,[3] who reported that inert materials such as proteins and salts of heavy metals, when combined with pituitary extracts, produce this augmentation phenomenon. Thus, it appears likely that the contained serum proteins, rather than any hormone, are responsible for the augmenting action of the mare serum.

The present work was undertaken in order to determine further the possibility of augmenting mare gonadotropic hormone through the use of pituitary factors. The mare hormone used was not taken from the blood serum but from the endometrium where it occurs in its greatest concentration. The pituitary preparation used was an acetone-ammonia extract[4] of sheep hypophysis, and its activity indicated that it contained primarily the luteinizing principle. The test animal throughout this work was the 21-day-old immature female mouse. Single injections of the pregnant mare gonadotropic hormone were made and the animals autopsied 5 days later; the pituitary factor, however, was administered in 3 doses given at 48-hour intervals, and the animals were sacrificed 5 days after the first injection.

Injection of a given quantity (6 rat units) of pregnant mare hormone into test animals increased the weight of the ovaries so that their combined weight in each animal averaged 4.3 mg. The above mentioned pituitary extract, when administered alone, had no observed effect on the weight of the ovaries of the test mice. Since it has been demonstrated repeatedly that luteinizing hormone is ineffective in immature animals, these results would appear to be what we should expect. In order to determine whether or not this pituitary luteinizing principle would augment the action of the pregnant mare hormone, these 2 preparations were administered simultaneously. The average weight of the ovaries of animals thus

[1] Cole, H. H., and Hart, G. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 370.

[2] Saunders, F. J., and Cole, H. H., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 505; Fevold, H. L., Hisaw, F. L., and Greep, R., *Am. J. Physiol.*, 1936, **117**, 68.

[3] Maxwell, L. C., *Am. J. Physiol.*, 1934, **110**, 458.

[4] Catchpole, H. R., and Lyons, W. R., *Am. J. Anat.*, 1934, **55**, 167.

treated was 9.0 mg. This represents an increase of 109% in ovary weight.

In order to test whether this was a hormonal action or was simply due to the presence of proteins, the sheep pituitary extract was inactivated by heating at 100°C. for 25 minutes. Using doses equal to those previously injected, the heated material was administered to test animals simultaneously with pregnant mare hormone, and it was found that the heated hypophyseal extract had lost its ability to augment the action of the pregnant mare hormone. These data tend to indicate that the augmentation obtained by using unheated pituitary extract is dependent upon hormone activity rather than upon the presence of inert proteins. However, since heating is known to produce profound chemical changes in proteins, further experiments, which would control this factor, seemed advisable. Therefore, an extract of muscle, which had the same concentration of proteins as that contained in the pituitary solution, was prepared, and this was injected into test mice simultaneously with pregnant mare hormone. Ovary weights indicated that the muscle extract is incapable of augmenting the gonadotropic material from the pregnant mare.

It is of interest to note that, although inert materials such as proteins and salts of heavy metals are able to augment the gonadotropic hormone from the pituitary gland, our data seem to indicate that these inert materials will not augment the endometrial gonadotropic hormone of the pregnant mare. This is in accord with the work of Saunders and Cole,[2] who state that the addition of $ZnSO_4$ to pregnant mare serum has no effect in augmenting the action of the latter substance. In order to explain the above phenomena adequately and correlate them with other recently published work, it is obvious that further information concerning the underlying mechanism of augmentation is essential. Experiments which may throw some light upon this problem are now being performed in this laboratory.

Summary. The action of the gonad-stimulating hormone obtained from the endometrium of the pregnant mare is augmented by pituitary extracts containing primarily the luteinizing hormone. This augmentation is not brought about by using inactivated pituitary material or extracts of muscle. Thus, augmentation obtained in this experiment is dependent on hormonal action and is not produced by the use of the inert materials tested.