trauma found in the experiments suggested investigation to determine if testosterone had an inhibiting effect on formation of deciduoma when these would normally occur. Six rats were taken on the first day of lactation and given 500 γ testosterone daily. By the fourth day the litters of 2 of these rats had died, though the mammary gland was found to be well developed and secreting, and these 2 animals were removed from the group. On the fifth day biopsy specimens of the uterus were taken and the uterine horn slit in the remaining 4 rats. The biopsy specimen showed the uterus to be in the state of second progestational proliferation, with a lower epithelium than is usual in this condition. Five days later the rats were killed. Histological examination of the slit uterus showed in 3 cases typical deciduomata and in the fourth case a typical endometrial mole. The animal in which mole formation occurred failed to rear its litter. Seven young were born and in 6 days the number was reduced to 3, and in 7 days to *nil*. It is suggested, though direct proof is lacking, that the animal was passing, at the time trauma was performed, into a state of post-lactation oestrus, and that the oestrin in circulation at this time resulted in formation of the endometrial mole. This chance occurrence affords evidence that, in addition to failing to inhibit deciduoma formation, testosterone does not prevent the formation of an endometrial mole.

Summary. From traumatization experiments on the rat uterus, it is concluded that the action of testosterone* differs qualitatively both from that of progesterone and from that of oestrogens.

9804

Hemolytic Properties of Indol.

ERIC PONDER.

From the Biological Laboratory, Cold Spring Harbor.

Rhoads and his collaborators[1, 2, 3] have recently established that the administration of indol to dogs on a deficient (Goldberger) diet results in a severe anemia which can be cured by adding the lacking

* The author is indebted to Dr. Erwin Schwenk of the Schering Corporation for the testosterone used in these experiments.

1 Rhoads, C. P., and Barker, W. H., *J. Exp. Med.*, 1938, **67**, 267.

2 Rhoads, C. P., and Miller, D. K., *J. Exp. Med.*, 1938, **67**, 273.

3 Rhoads, C. P., Barker, W. H., and Miller, D. K., *J. Exp. Med.*, 1938, **67**, 299.

factors to the diet, and have further shown that this anemia is the result of a hemolytic process rather than of the lack of a growth factor required for normal marrow activity. Because of these observations, the *in vitro* hemolytic properties of indol are of considerable interest, and these have not hitherto been described.

1. Indol is a weak hemolysin, a system containing 1.6 cc. of 1 mM/1. indol in 1% NaCl (this is a virtually saturated solution, and has a pH of 6.3), together with 0.4 cc. of a rabbit red cell suspension with $2.5(10^8)$ cells per cc., showing complete lysis in about 30 min. at 25°C. Half this concentration, however, does not show complete lysis for many hours. The lytic effect on the cells of man and of the dog are very similar. As in the case of most lysins, the lytic effect is strongly inhibited by serum or plasma, and, again as in the case of most lysins, the hemolysis is preceded by a discsphere transformation. In a saturated solution of indol, this occurs almost immediately.

2. Indol is a powerful accelerator of lysis by saponin, the value of (R-1)/c, which measures the accelerating power, being —0.60; this means that indol is about 24 times as acceleratory as benzene (millimol for millimol), and about as potent as iodobenzene. The acceleration of taurocholate hemolysis is smaller, *viz.*, —0.20, or about 8 times that of benzene. These values are at 25°C.

3. Like the effect of benzene and many of its derivatives, the effect of indol on the red cell membrane seems to be a double one. If the cells are brought into contact with the indol, and rapidly washed free of it, their resistance will be found to be scarcely decreased at all; if allowed to stand for some time in contact with the indol, on the other hand, (*e. g.*, 5 min. in 0.2 mM/1. indol), their resistance will be found to be considerably decreased. Sufficiently long contact with greater concentrations results in lysis by the indol *per se*, a clear indication of a direct effect on the cell membrane. The effect of the accelerator is therefore two-fold. In its presence, red cells immediately become more susceptible to the action of lysins, perhaps because the accelerator forms a new phase at the cell surface, and so alters the partition or rate of concentration of the lysin, and this phase of the action of the accelerator is reversible by washing; with time, however, the accelerator reacts irreversibly with the components of the cell surface, even to the point of producing lysis if the concentration is great enough, and the time long enough.

4. Indican, a conjugated form of indol, is neither lytic nor acceleratory; in fact, it is an inhibitor of saponin hemolysis. From a physico-chemical standpoint, however, the water-soluble indican

is very different from the comparatively insoluble indol, and so this result is not unexpected.

These results have a considerable bearing on the way in which the lytic process observed by Rhoads and his coworkers may be brought about, for the flooding with indol of the blood stream of a dog on a deficient diet is equivalent to the flooding of the blood stream with a weak lysin, or, perhaps more significantly, with a powerful accelerator for whatever intravascular lysins may be present. It is true, of course, that the *in vivo* effect of indol will be much less than the *in vitro* effects, partly because no great concentration of indol can occur in the blood stream, and partly because of the great inhibitory effects of the serum proteins and the great number of red cells present. The kinetics of *in vivo* lysis, acceleration, and inhibition, are at present completely unknown, so it is impossible to say exactly what the effect of establishing a *continually maintained* concentration of indol in the blood stream would be; it is safe to say, however, that the result of continually maintaining an accelerator, even in small amounts, in the blood stream would be to hasten any normal lytic process occurring there, and would, therefore, in the long run, tend to bring about an anemia.

9805

Ultracentrifugal Isolation of High Molecular Weight Proteins from Broad Bean and Pea Plants.

HUBERT S. LORING, H. T. OSBORN AND RALPH W. G. WYCKOFF.*

From the Rockefeller Institute for Medical Research, Princeton, New Jersey.

Characteristic high molecular weight proteins have been isolated by ultracentrifugation from the juices of plants diseased with several of the less stable viruses.[1] Various lines of evidence indicate that virus activity is a property of these high molecular weight proteins. The viruses thus far studied are easily transmitted by mechanical means. Certain viruses, however, require specific insect vectors and are difficult to transmit by ordinary mechanical methods. We have made an ultracentrifugal examination of infectious juice containing such a virus, choosing for the purpose pea mosaic (pea

* Now at Lederle Laboratories, Inc., Pearl River, New York.

1 Stanley, W. M., and Wyckoff, R. W. G., *Science*, 1937, **85**, 181; Loring, H. S., and Wyckoff, R. W. G., *J. Biol. Chem.*, 1937, **121**, 225.