ment holds for all the room temperatures used and is true immediately after the room temperature is changed and after a period for adaptation has elapsed. The maximal differences between the fore and hind limbs occurred at room temperatures between 16° and 20°C. and tended to decrease as the temperature became colder or warmer. After a period of 30 minutes in a room temperature of 30°C., the temperatures in the fore and hind limbs were essentially equal (confirmation of Reichert[11]).

These experiments do not support a reflex control of the blood vessels of the skin *via* the dorsal roots, as evidenced by skin temperature in the cat. With the thoracolumbar sympathetic innervation bilaterally removed, unilateral section of the dorsal roots does not produce a difference in the skin temperatures on the 2 sides under the experimental conditions we have used. We believe that the lower skin temperatures which Hinsey reported for deafferented limbs are to be attributed to an over-action of the vasoconstrictor mechanism in the absence of some inhibitory control from the somatic afferent supply to the respective limbs. We realize that, if there are afferent neurones situated in the sacral chain ganglia such as Schwartz[12] has reported in the stellate and lumbosacral chain ganglia of the cat, they were not removed in our experiments. If the vasomotor effects which Rosenblueth and Cannon[13] have described in sympathectomized animals are due to dorsal root efferent conduction, they were not reflected in the skin temperatures of the foot-pads as we recorded them.

7684 C

Concerning Gonadotropic Substances in Mare Serum.

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Evans *et al.*[1] have shown that there is a wide distribution of a substance in the urine and blood serum of mammals which resembles prolan, in that when it is mixed *in vitro* with the pituitary synergist the effect upon the ovary of the immature rat is enhanced. Evidence

[11] Reichert, F. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 473.

[12] Schwartz, H. G., *Am. J. Physiol.*, 1934, **109**, 593.

[13] Rosenblueth, A., and Cannon, W. B., *Am. J. Physiol.*, 1934, **108**, 599.

[1] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 561.

that a similar prolan-like substance is present in the blood serum of the non-pregnant and pregnant mare is given in Table 1.* Further the data indicate that the prolan-like substance is distinct from the substance in the blood serum of the pregnant mare which is so highly effective in stimulating the gonads when given alone. Following our nomenclature in previous publications we will term the latter substance the gonad-stimulating hormone.

TABLE I.

Response of Immature Rats to Mare Serum Alone and to the Combination of Mare Serum and Pituitary Synergist. The Rat Dose of Synergist Was in Every Instance 2 mg.

Serum taken after infertile mating days	Serum	Serum alone			Serum plus synergist		
		Serum injected	No. of rats	Av. wt. of 2 ovaries	Serum injected	No. of rats	Av. wt. of 2 ovaries
		cc.		mg.	cc.		mg.
8	N1	10	4	17*	2	12	26‡
11	N7	—	—	—	2	6	37‡
16	N2	10	4	18*	2	6	42‡
23	N3	10	4	19*	2	6	49‡
31	N4	10	4	19*	2	6	42‡
37	N5	10	4	19*	2	6	26‡
41	N6	10	4	14*	2	6	42‡
Average for sera of non-pregnant mares				18*			36‡
175 days pregnant	P1	2	6	21†	2	6	35‡
165 " "	P2	2	6	23‡	2	6	37‡
Average for sera of mares at mid-pregnancy				22			36‡
80 days pregnant	7	0.04	6	22‡	0.04	6	21‡
80 " "	7	0.08	6	24‡	0.08	12	37‡
80 " "	8	0.08	6	25‡	0.08	6	25‡
Average for sera of mares in early pregnancy				24‡			28‡

*Ovaries of all rats in the group were infantile.
†Ovaries of 3 of the 6 rats had ripe follicles or corpora.
‡Ovaries of all rats in the group contained ripe follicles or corpora.

Referring to Table 1 it may be seen that equivalent amounts of non-pregnant and mid-pregnancy mare serum are equally effective in stimulating the gonads of immature rats when combined with the pituitary synergist. The amount of gonad-stimulating hormone present in the 2 types of sera differs greatly. Two cc. of the mid-pregnancy serum when given alone induces follicular development and luteinization whereas 10 cc. of the non-pregnant serum produces no response. In fact, we have shown previously that doses of 60 cc. of non-pregnant serum are without response on the immature rat. Thus it is clear that there is no relationship between the response

* The synergist was prepared and administered according to the method of Evans *et al.*[2] The average ovarian weight of 11 rats receiving 2 mg. of synergist alone was 22 mg.

[2] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 545.

of the serum when given alone and the response of serum plus synergist. This fact is further demonstrated when the response of early pregnancy blood serum highly potent in gonad-stimulating hormone is compared to non-pregnant serum. The amount of early pregnancy serum injected contained 2 to 4 rat units of gonad-stimulating hormone while the non-pregnant serum contained none. Still the response of the early pregnancy serum plus synergist was less than the non-pregnant serum plus synergist. These results appear to us to be most easily explained by assuming that non-pregnant mare serum contains a single recognizable gonadotropic substance which is only effective when mixed *in vitro* with pituitary synergist and that pregnancy serum contains 2 substances—one effective by itself or possibly in conjunction with the pituitary of the recipient —the other effective only when mixed *in vitro* with the pituitary synergist.

Table 1 shows an apparent correlation between the time following an infertile mating and the response elicited in the ovaries of immature rats by the serum when combined with synergist. Further tests would have to be made to demonstrate that the increasing ovarian response is related to pseudo-pregnancy which itself has not yet been demonstrated in the mare.

We have made no attempt to determine the identity of these substances in mare serum with gonadotropic substances described by other workers. Hisaw and his associates have held the view, which has become increasingly popular, that the pituitary contains two gonadotropic substances, a "follicle-stimulating" and a "luteinizing" substance. They[3] were the first to give conclusive proof that the pituitary contains 2 gonadotropic substances. Our results presented above could be explained by assuming that the "luteinizing" substance is present both in non-pregnant and in pregnant mare serum and the "follicle-stimulator" only in the pregnant serum. In fact, Hisaw and his associates (personal communication) report that the serum of the pregnant mare contains these 2 substances. It seems very probable, however, that the recent finding of Smith *et al.*[4] in regard to the remarkable specificity of follicle-stimulating urine upon the seminiferous tubules of the hypophysectomized male rat and similar findings of Evans *et al.*[5] in regard to the hypophyseal

[3] Fevold, H. L., Hisaw, F. L., and Leonard, S. L., *Am. J. Physiol.*, 1931, **97**, 291.

[4] Smith, P. E., Engle, E. T., and Tyndale, H. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 745.

[5] Evans, H. M., Pencharz, R. I., and Simpson, M. E., *Endocrinology*, 1934, **18**, 607.

synergist may lead to a modification of views pertaining to the nature of the gonadotropic hormones.

7685 C

Hemolysins to which Reticulocytes are Especially Vulnerable in the Plasma of Primary Anemia.

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Ucko[1] has described a saponin-like substance present in normal plasma but in much larger amounts in active cases of primary anemia. It is more toxic to the reticulocytes than to the mature cells. It seemed to us that the presence of such a substance, rather than a structural defect in the reticulocytes of patients with primary anemia, might explain the fall of 30-60% in the reticulocyte count on incubating sterile defibrinated blood of active cases of primary anemia.[2] Blood from normal persons, from secondary anemia cases, or from cases of primary anemia during remission does not have a fall in reticulocyte count when incubated in the same way.[2, 3]

We therefore took blood from 4 active cases of primary anemia just before starting intensive parenteral liver therapy and preserved the plasma on ice for 4-7 days. Washed cells obtained at the height of the reticulocyte rise were then mixed with twice their volume of fresh plasma, and also, in the same proportion, with plasma obtained before treatment. These tubes were incubated at 37° C. All the blood was handled with sterile technique and counts were made only if there was no bacterial growth on incubation.

In one case, the mildest and least jaundiced (Van den Bergh, indirect, 1.25 units), the blood taken during the active stage had to be transported and cells and plasma were not separated until several hours after being drawn. This patient had 2.4 million red cells per cu. mm. before treatment and in this case the reticulocyte count in the tubes containing pre- and post-treatment plasma was the same after 24 hours incubation. The other 3 patients all had counts under 1.4 million and indirect Van den Berghs of 1.5 to 3 units before

[1] Ucko, H., *Z. f. klin. Med.*, 1931, **118**, 22.

[2] Buckman, T. E., and MacNaugher, E., *J. Med. Research*, 1923, **44**, 61.

[3] Mermod, C., and Dock, W., *Arch. Int. Med.*, in press.