the animal succumbed to infection. On examining the lesions produced, one could observe normal phagocytic activity and numerous organisms within the tissue of the lesions produced. Feinstone, Bliss, Ott and Long[3] present evidence indicating that the activity of Neoprontosil depends on its reduction to sulfanilamide *in vivo*.

Gye and Cramer[4] found that ionizable salts of calcium inoculated together with washed spores of *Cl. welchii* or *Cl. tetani* led to the development of the corresponding infections in their fatal form, while washed spores alone did not lead to death. Fildes[5–7] thinks that there must be some definite stimulus to vegetation in the tissues injected by calcium salts, and suggests that this stimulus is probably the result of diminished oxygen tension. He showed further that the injection of solutions of calcium chloride lead to the production of localized areas of oxygen deficiency. We do not know if Neoprontosil in this case acts in a similar way.

Summary—We were unable to protect mice from M.L.D. of *Cl. welchii* by intramuscular injection of Neoprontosil. The intramuscular injection of washed cells of *Clostridium welchii* with Neoprontosil in mice led to the development of a fatal infection.

11518

Blood Progesterone During Sexual Cycle of *Macaca rhesus;* Quantitative Assay.

INÉS L. C. DE ALLENDE.* (Introduced by G. W. Corner.)

From the Department of Anatomy, The University of Rochester, School of Medicine and Dentistry.

At the suggestion of Professor George W. Corner, the presence of progesterone in the blood during the menstrual cycle of the monkey, *Macaca rhesus,* has been studied and an effort made to

3 Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hospital,* 1938, **62**, 565.

4 Gye, W. E., and Cramer, W., *Sixth Sci. Rep. Imp. Cancer Res. Fund,* 1919, pp. 40-57.

5 Fildes, P., *Brit. J. Exp. Path.,* 1927, **8**, 387.

6 *Ibid.,* 1929, **10**, 151.

7 *Ibid.,* 1929, **10**, 197.

* Fellow of the Argentina Association for the Advancement of Sciences. This work was made possible by a grant made to Dr. George W. Corner through the University of Rochester by the John and Mary R. Markel Foundation.

establish the curve of its variation. For this purpose the results recently obtained by McGinty, Anderson and McCullough[1] provide a sensitive qualitative test and within limits (Haskins)[2] make possible a rough quantitative assay.

Method. Samples of blood were taken every 3 days and the serum extracted with ether. The ether extract was treated with 0.2 N sodium hydroxide and the ether-soluble fraction evaporated and the residue dissolved in peanut oil. A series of dilutions was made in such a way that each 0.1 cc of oil contained the ether-soluble fraction of 1, 2, 3 and 4 cc of normal serum, respectively. The hormone was tested by the method of local intrauterine injections of de Mussio-Fournier, Albrieux, Morato, and Grosso.[3] Immature rabbits of an average weight of 710 g were injected daily with 25 I.U. of estrogen (Amniotin) for 6 days. On the 7th day operation was performed under anesthesia, and 2 segments 2 cm long were isolated between ligatures in each uterine horn. Three of these were injected with the dilutions which contained the ether-soluble fraction of the different quantities of normal serum, and the fourth with peanut oil as a control. Autopsies were done at 72 hours after the operation and the segments of the uterus were examined histologically. The degree of endometrial proliferation was evaluated according to the scale of McPhail.[4]

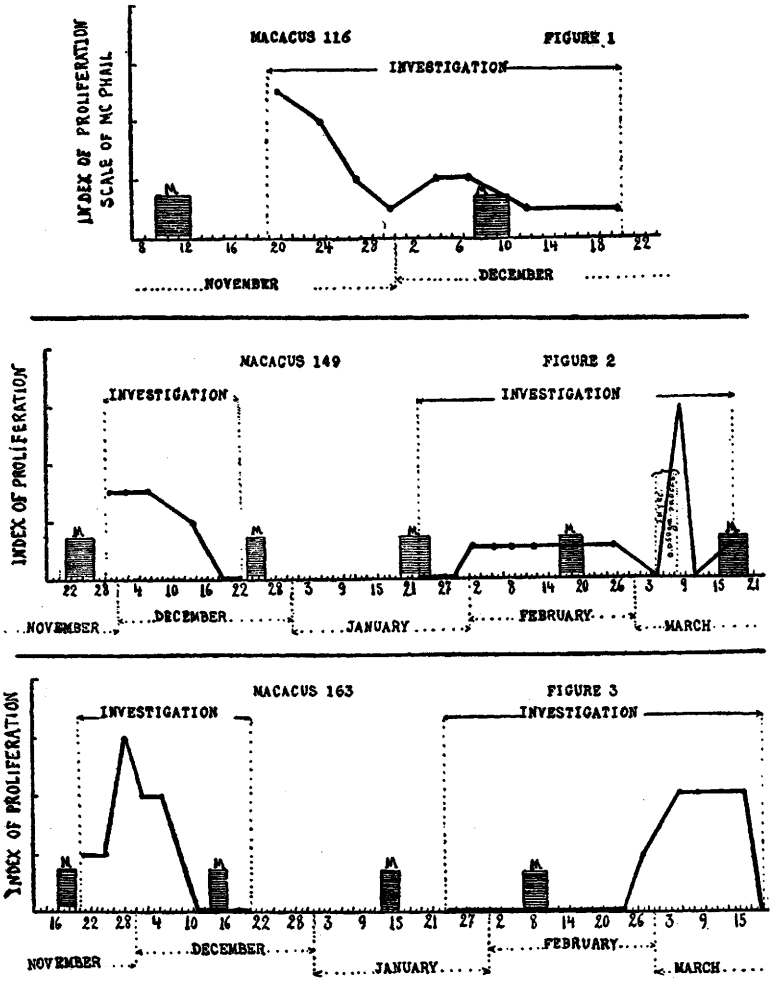
Results. Figs. 1, 2 and 3 show the results obtained in 3 Rhesus monkeys of the same age and weight. The curve represents the variations of the degree of proliferation of the endometrium of the immature rabbit obtained with 1, 2, 3 and 4 cc, respectively, of blood serum, studied every 3 days. The maximum reactions corresponded to the value +++ on the scale of McPhail and the minimum measured between 0 and +. In 60% of those cases in which the serum extract gave a positive reaction in the segments in which it was injected, the segment injected with peanut oil also showed a positive reaction between ++ to +++ and +, probably because the hormone circulating in the blood stream is concentrated in the peanut oil. For this reason, in the second series the use of peanut oil as a control was abandoned. In the first study, made during the November-

[1] McGinty, D. A., Anderson, L. P., and McCullough, M. B., *Endocrinology*, 1939, **24**, 829.

[2] Haskins, Arthur L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 624.

[3] Mussio Fournier, J. C., Albrieux, A. S., Morato, J., and Grosso O., *Bull. de l'Acad. de Med. Paris*, 1938, **120**, 273; *Rev. de Obstet. e Ginec. de Sao Paulo*, 1938, **3**, 203; *Endocrinology*, 1939, **24**, 515.

[4] McPhail, M. K., *J. Physiol.*, 1935, **83**, 145.



Curves of progesterone in the blood during the sexual cycle of *Macaca rhesus.*

December cycle, the curve shows in monkeys 116 and 163 (Fig. 1 and 3) a maximum reaction of +++ and ++ to +++, respectively, on the 10th and 11th day of the cycle, while in monkey 149 (Fig. 2) it is maintained at the same level (+ to ++) during this first half of the cycle. In the 3 animals the curve drops gradually in the second half of the intermenstrual period; but while in 2 of them (Fig. 2 and 3) it becomes negative 3 and 5 days before the next menstruation, in monkey 116 (Fig. 1) it reaches a proliferative reaction equal to + the day before the catamenial flow.

The great similarity of results in this first cycle was not observed when the investigation was repeated in animals 163 and 149.

Monkey 163, which showed all negative reactions in the second half of the January-February cycle (Fig. 3) maintained this negativity, during the first and part of that which would have constituted the second part of the February-March cycle, which was incomplete due to the failure of menstruation in the latter month. If we assume that menstruation would have occurred between the third and the seventh of March, the maximum reaction (++) was observed precisely in this period and the curve maintains this maximum during what would have been the first half of the following cycle, thus being in accord with the first study made. In monkey 149 (Fig. 2) weak reactions were obtained at the end of the first and during the second half of the January-February cycle and moderate reactions in the first part of the February-March cycle.

In order to control the procedure used we injected this animal with 0.010 g of progesterone daily for 5 days. Blood taken on the sixth day gave a +++ reaction of proliferation with 2 cc of serum, successive samples being negative.

Quantitative Assay. If we use the figures found by McGinty to evaluate the uterine proliferative reactions we should be able, by trying measured doses, to calculate indirectly the quantity of progesterone existing in the blood at any given moment. If as stated by this author, ++ to +++ reactions in the uterus of the rabbit are produced by doses of progesterone which vary from 5 to 0.5 γ, when we obtain in our case the same reaction with 2 cc of serum, we may deduce that each cubic centimeter of blood serum contained, at the moment it was taken, between 2.5 and 0.25 γ of progesterone.

Conclusions. 1. The presence of progesterone was demonstrated in the blood of the normal *Macaca rhesus*. 2. The hormone in 1, 2, 3 and 4 cc of blood serum was studied every 3 days by means of local intrauterine injection in immature rabbits and the degrees of progestational reaction of the endometrium measured according to the scale of McPhail. 3. With this procedure it was possible to obtain the curve of its variations during a complete menstrual cycle in 3 Rhesus monkeys of the same age and weight. 4. In 2 of the animals the curve shows its maximum (++ to +++ reaction) on the 10th and 11th day of the menstrual cycle respectively, while in the third it was maintained at a constant level (+ to ++ reaction) during the first half of the cycle. In the 3 monkeys the curve gradually fell during the second half of the menstrual cycle. 5. Studies repeated in 2 of the first animals, during 2 complete cycles, did not give such clear and definite results, perhaps because

depend more upon the batch of histamine mixture used than upon the individual animals. Recently, with increased experience in preparation of the material, reactions have been less frequent. In all animals, however, vomiting occurred some time during the course of injections and when not associated with the immediate reaction took place several hours after the injection. The vomitus usually contained free acid.

These 7 cats receiving the histamine beeswax mixture were sacrificed at periods ranging from 3 to 25 days after beginning the injections, the total amount of histamine given ranging from 60 to 480 mg. At necropsy there were erosions or acute ulcers of the stomach or duodenum, or both, in all animals. Two cats had lesions of the duodenum only, 2 had lesions of the stomach only, and 3 had lesions of both stomach and duodenum. In 3 animals there were perforated ulcers, two in the duodenum and one in the stomach. Gastric lesions were limited to the antrum. It seemed obvious that the animals had been sacrificed at various stages of ulcer formation.

Comment. Histamine in beeswax prepared according to the method of Code stimulated a sustained copious flow of gastric juice containing free acid when injected intramuscularly into cats. Repeated injections of histamine in saline solution have been reported as failing to produce ulceration in the gastro-intestinal tract in the dog[2] and also in the cat.[3] Repeated single daily doses of histamine in beeswax in this study were effective in the cat in producing erosions and all stages of ulceration including acute perforation. In one dog tested, ulceration occurred in the duodenum.† These findings suggest the importance of the gradual liberation of histamine from beeswax in maintaining a constant and fairly uniform stimulation of gastric secretion, as opposed to the intermittent stimulation afforded by periodic injections of histamine in watery solution.

---

2 Orndorff, J. R., Bergh, George S., and Ivy, A. C., *Surg. Gynec. Obstet.*, 1935, **61**, 162.

3 Heinlein, H., and Kastrup, H., *Z. f. d. ges. exp. Med.*, 1938, **102**, 517.

† Since this paper was written intramuscular implantation of histamine has been done in an additional cat and in another dog. Both animals were killed and autopsied when it was apparent that they were ill. The cat had a large ulcer in the fundus 12 days after the administration of histamine was begun. The dog had a small ulcer .5 cm in diameter in the first portion of the duodenum 4 days after histamine administration was begun.

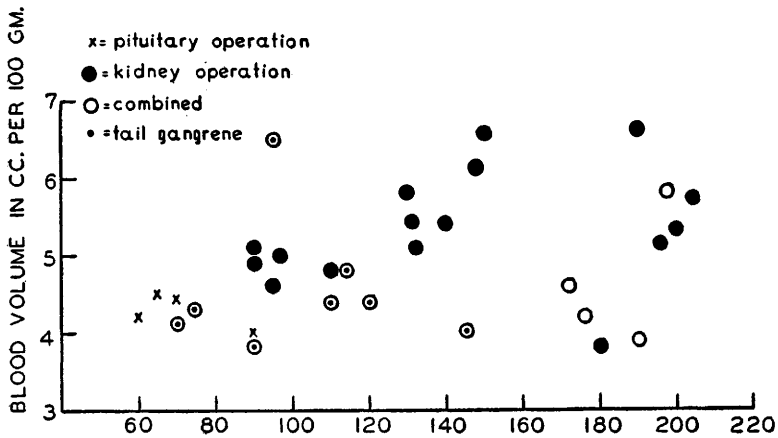


FIG. 1.

Blood pressure of the normal rats, under nembutal anesthesia, will not exceed 140 mm of mercury when measured by this method, and less than 5% of the animals will show pressures in excess of 120. Blood volume, expressed in cc per 100 g body weight ranges from 4.0 to 5.0 cc in normal animals weighing over 160 g while the upper limit in animals weighing less than 160 g is 5.3. All animals used in this study weighed well over 160 g.

It is seen in Fig. 1 than in half of the 16 animals, represented by solid dots, the blood pressure was 140 or over. In all rats with blood pressures of 140 or higher the blood volume was 5.1 or more except in one case, a female with a blood pressure of 180 and a blood volume of only 3.8. This animal may have been pregnant and, if so, should not be included in the series. Some elevation of blood volume occurred in 3 animals with blood pressures over 130, while the remaining animals with definitely normal blood pressures tended to have blood volumes within the normal range.

Procedure 2. The posterior lobe and half the anterior lobe of the pituitary were resected in 4 animals by the usual parapharyngeal route. All promptly developed typical diabetes insipidus. Blood pressure and blood volume measurements, made 7 to 10 days later, are charted as crosses in Fig. 1. All were normal.

Procedure 3. Fourteen animals survived the combined pituitary and kidney operations as described in procedures 1 and 2. The operations were performed in 3 stages a week apart, the usual order being subtotal nephrectomy, pituitary operation, nephrectomy. The first blood pressure measurement was made one week after the last

operation. Only 2 of the 14 animals were hypertensive. This measurement was repeated one week later, when 7 out of 14 were found to be hypertensive. Thus the appearance of the hypertension was somewhat delayed. At this time blood volume was measured in 12 animals (one died and in one there was a technical error in the intravenous injection of the dye). The results are shown in Fig. 1 as open circles. Blood volume was increased in only 2 animals, one of which was hypertensive while one was not. Blood volume was normal in 4 animals with hypertension.

As an unexpected finding, certain animals undergoing the combined operations as outlined in procedure 3 developed gangrene of the tail, which may be described as moist in contrast with the dry gangrene seen after ergotamine poisoning. This occurred in 8 of the 13 surviving animals, 12 of which are charted in Fig. 1. Rats with gangrene are indicated by dots placed centrally in the open circles. The 13th animal had a blood pressure of 163, no tail gangrene, but blood volume determination failed and, therefore, it is not charted. It is apparent that there was a tendency for animals developing hypertension to escape the gangrene.

Comment. While the number of animals is too small to permit absolute conclusions, certain tendencies are obvious. The increase in blood pressure occurring in rats with only about half of one kidney remaining is associated, as a rule, with increased blood volume. In the absence of the posterior lobe of the pituitary, such elevation in blood volume does not occur, but the vascular hypertension is unaffected. This may mean that the increased blood volume is maintained through the mediation of the posterior lobe of the pituitary, or, more likely, that in the presence of a marked continuous diuresis an increased blood volume is more difficult to maintain. The gangrene of the tail is difficult to explain. It has never occurred in several hundred cases where either the kidney or the pituitary operations were performed alone. It would appear that without a vascular hypertension the animal is unable adequately to maintain its peripheral circulation under the conditions detailed in procedure 3.

Summary. Experimental hypertension may develop in the partially nephrectomized rat in either the presence or absence of the posterior pituitary. In the presence of the posterior pituitary 7 of 8 hypertensive rats showed an increase in blood volume whereas in the absence of the posterior pituitary 4 of 5 hypertensive rats showed a normal blood volume.